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A U T H O R S .

	PAGE
BICKFORD. "The fibre dissociation produced by cooling human nerves"	159
CERQUA and SAMAAAN. "Cure of experimental renal hypertension"	113
COLLARD, MILLS, RUNDLE and SHARPEY-SCHAFER. "Thyrotropic hormone in the blood"	323
COPE. "The excretion of pregnandiol and the corpus luteum" ...	217
GARRY, WESTWATER and STIVEN. "A note on the serum sodium level in patients suffering from tuberculosis"	73
GLYNN and HIMSWORTH. "Experimental trinitrotoluene poisoning ; the effect of diet"	421
GRANT. "Observations on periarteritis nodosa"	245
HARRINGTON, POCHIN and SQUIRE. "A simplification of the Evans blue method of blood volume determination"... ..	311
HILL and PICKERING. "Hypertension produced in the rabbit by prolonged renin infusion"	207
HIMSWORTH and GLYNN. "Experimental trinitrotoluene poisoning ; the effect of diet"	421
HIMSWORTH and KERR. "Age and insulin sensitivity"	153
HIMSWORTH and KERR. "Insulin and alimentary hyperglycæmia in young normal subjects"	1
HIMSWORTH and KERR. "Insulin-sensitive and insulin-insensitive types of diabetes mellitus"	119
HIMSWORTH and KERR. "Pituitary like factors in the blood and urine of diabetic patients and of animals treated with pituitary extracts"	287
HOLLING. "Observations on the oxygen content of venous blood from the arm vein and on the oxygen consumption of resting human muscle"	103
KELLGREN. "On the distribution of pain arising from deep somatic structures with charts of segmental pain areas"... ..	35
KELLGREN. "Somatic simulating visceral pain"	303

	PAGE
KELLGREN. "Some painful joint conditions and their relation to osteoarthritis"	193
KELLGREN and LEWIS. "Observations relating to referred pain, visceromotor reflexes and other associated phenomena" ...	47
KELSALL, PICKERING and PRINZMETAL. "The assay of renin in rabbits with experimental renal hypertension"... ..	401
KERR and HIMSWORTH. "Age and insulin sensitivity"	153
KERR and HIMSWORTH. "Insulin and alimentary hyperglycæmia in young normal subjects"	1
KERR and HIMSWORTH. "Insulin-sensitive and insulin-insensitive types of diabetes mellitus"... ..	119
KERR and HIMSWORTH. "Pituitary like factors in the blood and urine of diabetic patients and of animals treated with pituitary extracts"	287
LEWIS. "Observations upon the vascular axon reflex in human skin, as exhibited by a case of urticaria, with remarks upon the nocifensor nerve hypothesis"	365
LEWIS. "Swelling of the human limbs in response to immersion in cold water"	349
LEWIS. "The adjustment of bloodflow to the affected limb in arteriovenous fistula"	277
LEWIS. "Trousseau's phenomenon in tetany"	361
LEWIS. "Venous pulsation in the orbit"	243
LEWIS and KELLGREN. "Observations relating to referred pain, visceromotor reflexes and other associated phenomena..."	47
MCGIBBON and MCMICHAEL. "Postural changes in the lung volume"	175
MCMICHAEL. "Hyperpnœa in heart failure"	19
MCMICHAEL. "A rapid method of determining lung capacity" ...	167
MCMICHAEL and MCGIBBON. "Postural changes in the lung volume"	175
MILLS, RUNDLE, SHARPEY-SCHAFER and COLLARD. "Thyrotropic hormone in the blood"	323
PICKERING and HILL. "Hypertension produced in the rabbit by prolonged renin infusion"	207
PICKERING, PRINZMETAL and KELSALL. "The assay of renin in rabbits with experimental renal hypertension"... ..	401

	PAGE
POCHIN. "Ocular effects of sympathetic stimulation in man" ...	79
POCHIN. "Oedema following ischæmia in the rabbit's ear" ...	341
POCHIN. "The mechanism of lid retraction in Graves' disease"...	91
POCHIN, SQUIRE and HARINGTON. "A simplification of the Evans blue method of blood volume determination"	311
PRINZMETAL, KELSALL and PICKERING. "The assay of renin in rabbits with experimental renal hypertension"	401
RUNDLE and WILSON. "Measurement of duction movements of the eye"	385
RUNDLE, SHARPEY-SCHAFER, COLLARD and MILLS. "Thyrotropic hormone in the blood"	323
SAMAAN and CERQUA. "Cure of experimental renal hypertension"	113
SCHIRE and SHARPEY-SCHAFER. "The effect of œstrogens on the urinary creatinine of castrate and menopausal women"...	185
SHARPEY-SCHAFER and SCHIRE. "The effect of œstrogens on the urinary creatinine of castrate and menopausal women"...	185
SHARPEY-SCHAFER, COLLARD, MILLS and RUNDLE. "Thyrotropic hormone in the blood"	323
SQUIRE. "An instrument for measuring the quantity of blood and its degree of oxygenation in the web of the hand"	331
SQUIRE, HARINGTON and POCHIN. "A simplification of the Evans blue method of blood volume determination"	311
STIVEN, GARRY and WESTWATER. "A note on the serum sodium level in patients suffering from tuberculosis"	73
WESTWATER, STIVEN and GARRY. "A note on the serum sodium level in patients suffering from tuberculosis"	73
WILSON and RUNDLE. "Measurement of duction movements of the eye"	385

CONTENTS.

	PAGE
<i>No. 1 (Issued June 5th, 1939).</i>	
Insulin and alimentary hyperglycæmia in young normal subjects. By H. P. HIMSWORTH and R. B. KERR	1
Hyperpnœa in heart failure. By JOHN McMICHAEL	19
On the distribution of pain arising from deep somatic structures with charts of segmental pain areas. By J. H. KELLGREN	35
Observations relating to referred pain, viscero-motor reflexes and other associated phenomena. By T. LEWIS and J. H. KELLGREN	47
"A note on the serum sodium level in patients suffering from tuber- culosis." By J. O. WESTWATER, D. STIVEN and R. C. GARRY	73
Ocular effects of sympathetic stimulation in man. By E. E. POCHIN	79
The mechanism of lid retraction in Graves' disease. By E. E. POCHIN	91

No. 2 (Issued December 23rd, 1939).

Observations on the oxygen content of venous blood from the arm vein and on the oxygen consumption of resting human muscle. By H. E. HOLLING	103
Cure of experimental renal hypertension. By S. CERQUA and ADLI SAMAAN	113
Insulin-sensitive and insulin-insensitive types of diabetes mellitus. By H. P. HIMSWORTH and R. B. KERR	119
Age and insulin sensitivity. By H. P. HIMSWORTH and R. B. KERR	153

	PAGE
The fibre dissociation produced by cooling human nerves. By REGINALD G. BICKFORD	159
A rapid method of determining lung capacity. By JOHN McMICHAEL	167
Postural changes in the lung volume. By J. McMICHAEL and J. P. McGIBBON	175
The effect of oestrogens on the urinary creatinine of castrate and menopausal women. By E. P. SHARPEY-SCHAFER and I. SCHRIRE	185
Some painful joint conditions and their relation to osteoarthritis. By J. H. KELLGREN... ..	193
Hypertension produced in the rabbit by prolonged renin infusion. By JANET R. HILL and G. W. PICKERING... ..	207

No. 2 (Issued October 30th, 1940).

The excretion of pregnandiol and the corpus luteum. By CUTHBERT LESLIE COPE	217
Venous pulsation in the orbit. By T. LEWIS	243
Observations on periarteritis nodosa. By R. T. GRANT	245
The adjustment of bloodflow to the affected limb in arteriovenous fistula. By THOMAS LEWIS... ..	277
Pituitary like factors in the blood and urine of diabetic patients and of animals treated with pituitary extracts. By H. P. HIMSWORTH and R. B. KERR	287
Somatic simulating visceral pain. By J. H. KELLGREN	303
A simplification of the Evans blue method of blood volume determina- tion. By C. R. HARINGTON, E. E. POCHIN and J. R. SQUIRE ...	311
Thyrotropic hormone in the blood. By H. B. COLLARD, F. H. MILLS, F. F. RUNDLE and E. P. SHARPEY-SCHAFER	323
An instrument for measuring the quantity of blood and its degree of oxygenation in the web of the hand. By J. R. SQUIRE... ..	331

No. 4 (Issued December 15th, 1942).

Oedema following ischæmia in the rabbit's ear. By E. E. POCHIN ...	341
Swelling of the human limbs in response to immersion in cold water. By THOMAS LEWIS	349
Trousseau's phenomenon in tetany. By THOMAS LEWIS	361
Observations upon the vascular axon reflex in human skin, as exhibited by a case of urticaria, with remarks upon the noci- fensor nerve hypothesis. By THOMAS LEWIS	365
Measurement of duction movements of the eye. By F. F. RUNDLE and C. W. WILSON	385
The assay of renin in rabbits with experimental renal hypertension. By G. W. PICKERING, M. PRINZMETAL and A. R. KELSALL ...	401
Experimental trinitrotoluene poisoning; the effect of diet. By H. P. HIMSWORTH and L. E. GLYNN	421

INSULIN AND ALIMENTARY HYPERGLYCAEMIA IN YOUNG NORMAL SUBJECTS.

By H. P. HIMSWORTH and R. B. KERR.

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IN 1936 a test was described by one of us (H.P.H. (8)) which, it was suggested, differentiated cases of diabetes mellitus into two types. It consisted in administering glucose orally and insulin intravenously as simultaneously as possible, and the basis of the differentiation was the degree to which alimentary hyperglycaemia was suppressed by the insulin. Cases in which the suppression was immediate and marked were classified as insulin sensitive diabetics, whilst cases in which this suppression was delayed and slight were classified as insulin insensitive diabetics. Early in the work with this test it became clear that, in some cases of diabetes, the degree to which insulin suppressed alimentary hyperglycaemia was influenced by the composition of the diet and, as might be expected, by the dose of insulin injected. It was therefore thought advisable to clarify the part played by each of these two factors in the test in normal subjects before attempting further interpretation of the data collected from diabetics.

Methods.

Experimental subjects. Four healthy young men whose ages ranged from 18 to 21 years were used for the investigation. As far as could be ascertained none had ever suffered from a serious illness and all denied a family history of diabetes. They were admitted to the ward of the hospital, where they lived under approximately standard conditions.

Diet. The diet prescribed was weighed and prepared in the diet kitchen* of the hospital and the complete consumption of the food was checked by the ward sister. There is no reason to believe that the diets were surreptitiously supplemented. Detection of non-adherence to the diet was possible when the subjects were taking a low-carbohydrate high-fat diet because ingestion of extra carbohydrate, which was naturally desired under this regime, was known to cause a diminution or disappearance of ketonuria. It is probable that if this unpalatable diet were adhered to the other more palatable diets were also observed. No test was performed until the subject had been taking the particular diet for at least one week.

* We are indebted to Miss E. Washington for her careful control of the diets.

Preparation for test. We would emphasise that the different tests were performed as carefully and under as strict conditions as are required for the estimation of the Basal Metabolic Rate.

No breakfast was taken on the day of the test and the last meal was at 6.30 p.m. the previous night. The subject went to bed at 8 p.m. and remained there until 9.30 a.m. next day. He was then transferred on a comfortable wheel chair to the laboratory next the ward, and after a rest of half an hour the test was begun. The subject remained in the chair throughout the experiment. The laboratory was heated to a comfortable temperature and by appropriate adjustment of blankets the subject was maintained comfortably warm. All tests were begun at 10.15 a.m.

At the commencement of the series of tests each individual was subjected to a series of mock experiments, the object of which was to habituate him to the experimental procedures used. Under the usual experimental conditions saline instead of insulin was injected intravenously, water flavoured with lemon and a little sugar, instead of the full dose of glucose, was given by mouth, and the full complement of blood samples was taken.

After two such mock experiments a real experiment was performed and this was repeated, at intervals of one or two days, until a constant result was obtained. The series of experimental observations were then begun.

Blood sugar estimations. These were performed by the Hagedorn-Jensen method in 0.1 c.c. of whole blood. The samples were taken from the puncture in the warm ear direct into the pipette. The puncture was made with a piece of glass capillary tubing which was bored into the lobe of the ear and removed a small plug of tissue. The puncture was made whilst the patient was in bed half an hour before the experiment commenced. To restart the flow of blood the capillary tube, after dipping into a powder of potassium oxalate crystals, was inserted rapidly into the puncture and withdrawn. Bleeding was stopped by gentle pressure with filter paper.

Three blood samples were taken immediately before the testing substances were given. Subsequent samples were taken at 5 mins., 10 mins. and at 10 minute intervals to the end of the first hour, and then at 15 minute intervals to the end of the third hour. The time of their collection was noted to the nearest quarter minute.

The tests. Two tests were used, the glucose tolerance test and the insulin-glucose test. In the former a dose of glucose was given by mouth, in the latter insulin was given intravenously and then the dose of glucose was immediately swallowed. In each case the resulting rise of blood sugar was measured. The dose of glucose was 30 g. per sq. m. of body surface; The dose of insulin varied. The glucose was dissolved in about 400 c.c. of cold tap water and was flavoured with citric acid and essence of lemon. The insulin was measured in an accurately graduated 1 c.c. "tuberculin" syringe. Doses of 4 units and more were given undiluted; in lower dosages it was diluted with normal saline for accuracy of measurement. The injections were made into the antecubital vein and to reduce the pain of the injection a

fine hypodermic needle (No. 20) was used. Danish Leo insulin (20 units per c.c.) was used, it having been found, in experiments on animals, that this insulin was practically free from the impurity which causes an initial hyperglycæmia on intravenous injection.

Measurement of the sugar tolerance and insulin-glucose curves. In a previous paper (7) a method was described by which changes in the level of the blood sugar, produced either by ingestion of glucose or injection of insulin, could be measured accurately and compared. This method consists in measuring the area traced out by the curve of blood sugar in the case of the glucose tolerance curve above, or in the case of the insulin depression curve below, the resting blood sugar level. The result is expressed in mg./min.. The only difficulty encountered in elaborating this method was to decide at what time the curve should be terminated, and previously on comparing a series of sugar tolerance curves it had been found satisfactory to terminate the areas at the shortest time that any curve of a particular series returned to the resting level. In Table I are given the results in a number of duplicate

TABLE I.

Table showing the constancy of the area demarcated by the sugar tolerance curve where the diet with a constant amount of carbohydrate is being taken.

Subject.	Diet.			Area of glucose tolerance in mg./mins.
	Carb.	Pret.	Fat.	
I.	100	80	218	6,600 6,500
II.	50	80	240	9,650 9,450 8,000
III.	50	80	240	18,600 15,900
A.	250 250 250	80 80 80	100 200 300	3,500 3,527 3,534
B.	55 660	88 90	202 42	11,960 13,090 5,590 5,400
C.	55 660	90 90	160 42	11,930 10,660 4,160 4,550
D.	56	87	255	10,890 11,100 10,750

The glucose tolerance areas were terminated at the shortest time taken by any glucose tolerance curve on the particular subject to return to normal. The interval between different glucose tolerance tests on the same diet varied from three days to nine weeks. The figures demonstrate that the greatest discrepancies between duplicate experiments are found when a low carbohydrate diet is being taken.

experiments on different subjects and it will be seen that by this method a surprising constancy in corresponding experiments is revealed.

In the present series of experiments, however, two new difficulties of measurement were encountered. Our object was to measure the effect of insulin in the presence of alimentary hyperglycemia, and this was done by measuring the area between the blood sugar curve after glucose alone and the curve after glucose + insulin. The first difficulty was to adjust the curves to start from the same resting blood sugar level for it is well known that the higher the blood sugar level the greater the action of insulin (3) (9) (6). Various methods were tried, but eventually it was decided to neglect the factor of fasting blood sugar level because, the resting blood sugar levels of any particular normal subject varying so little, the result on the subsequent blood sugar curve was practically negligible. We therefore adjusted all curves in one individual to the same resting level by algebraic addition of the difference between the value 100 mg./100 c.c. and the resting blood sugar level in any one experiment. The second difficulty was concerned with the time at which the area measurement should be terminated. It was frequently found that in the second hour the insulin-glucose curve rose to higher levels than the corresponding glucose tolerance curve and thus the area enclosed between the two became a negative value (Fig. 1). The significance of such

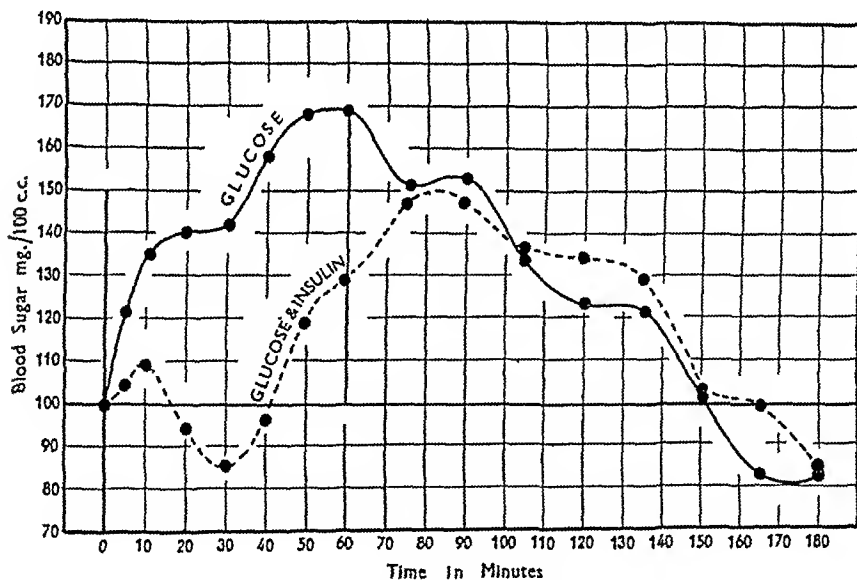


Fig. 1. The glucose tolerance curve (solid line) and the insulin-glucose curve (broken line) obtained from Subject I whilst taking the diet containing 100 g. of carbohydrate. Fasting blood sugar level before glucose tolerance test 98 mg./100 c.c., before insulin-glucose test 102 mg./100 c.c.. The test was commenced at 0 min.. After 102 min. the insulin-glucose curve rises higher than the glucose tolerance curve thus giving a negative value to the area enclosed between them. To avoid such negative areas measurements were terminated at 60 min. as indicated by the vertical line drawn to cut the two curves.

a value is obscure and it appears unreasonable to treat it by algebraic addition to the preceding positive area obtained in the first hour. For this reason the time of natural termination of the curves, at the shortest time, for any curve of a series to return to normal, had to be abandoned and it was ultimately decided to confine our measurements to the first 60 mins of the curve. This made it possible to avoid negative areas altogether. As a consequence of this curtailment to the first hour our figures for the difference between the glucose tolerance and the insulin-glucose curve measure the rate at which the injected insulin comes into action rather than its total effect.

For convenience of discussion the following abbreviated terms will be used. The area enclosed by the glucose tolerance curve above the resting level of blood sugar will be called the glucose tolerance area (G. area); the area enclosed between the glucose tolerance curve and the insulin glucose curve, which represents the action of the injected insulin, will be called the insulin area (I. area); the area below the insulin glucose curve, obtained by subtracting the insulin area from the glucose tolerance area, will be called the residual or insulin-glucose area (R. area).

Results.

1. *The effect of varying doses of insulin upon alimentary hyperglycæmia, the diet being constant.* In this series of experiments the test dose of glucose (30 g. per sq.m. body surface), the diet and the external factors were kept constant whilst the dose of insulin given in the insulin-glucose test was varied. After preliminary glucose tolerance and insulin-glucose curves had established by their constancy that the subject was habituated to the diet and experimental procedures, insulin-glucose tests with different doses of insulin were performed each at intervals of two or three days. All four subjects received diets of 2,680 calories but the composition varied. As it has already been shown (7) that the only dietetic factor affecting sugar tolerance or insulin sensitivity is the absolute amount of carbohydrate in the diet, the different diets can be designated by their carbohydrate content. Subjects II and IV received diets containing 50 g. of carbohydrate, Subject I 100 g., and Subject III 215 g. The dose of insulin used varied from 0 to 9 units, the insulin-glucose test with 0 units being, of course, the same as the glucose tolerance test. A few experiments with larger doses than 9 units were carried out but the results are not reported in this paper. With such large doses the depth of hypoglycæmia produced shortly after injection of the insulin is sufficient to provoke a compensatory return of the blood sugar to normal and thus to falsify the insulin-glucose curve.

The results of the experiments are expressed graphically in Fig. 2 where the insulin area in mg./min is charted against the corresponding dose of insulin. It will be seen that the curves showing the relationship between these two factors, although rising to different levels, are similar in type. The results show that the larger the dose of insulin the greater the insulin

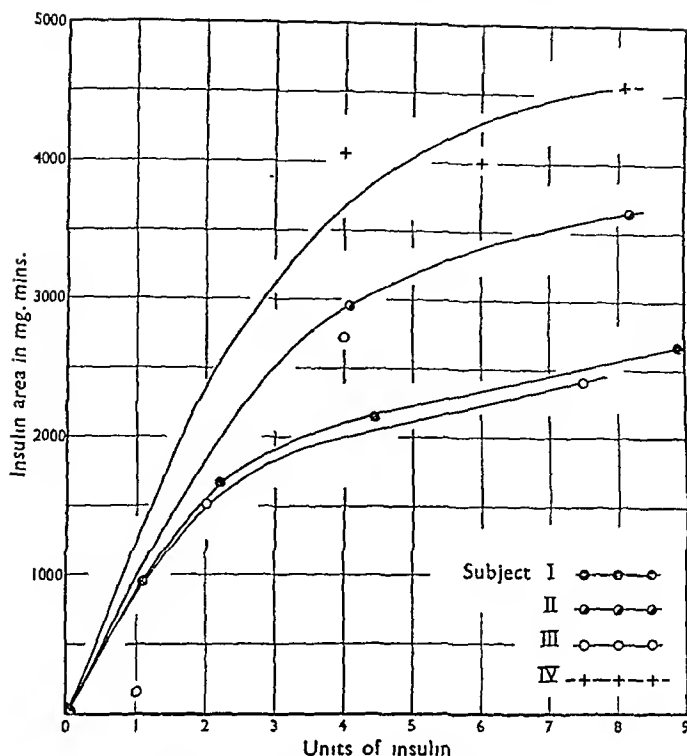


Fig. 2. Four curves showing the relationship in different subjects between the dose of insulin used in the insulin-glucose test and the degree to which it suppresses alimentary hyperglycæmia. Suppression of hyperglycæmia was measured by the insulin areas (glucose tolerance area—insulin-glucose area). Subject II and Subject IV were taking the diet containing 50 g. of carbohydrate; Subject I the diet containing 100 g. of carbohydrate; Subject III the diet containing 215 g. of carbohydrate.

area but that each successive increment of insulin produces a progressively smaller increase in insulin area.

The similarity of the curves suggests that the relationship between insulin dosage and insulin effect is of the same nature in different individuals, but that in each individual it is modified by certain other factors. In these experiments one such factor is the composition of the diet. The other factor that must be recognised is that of personal idiosyncrasy. As has been shown previously the proportional changes in the glucose tolerance curve in response to variations in the carbohydrate content of the diet are the same for all individuals, but the absolute changes in area are peculiar to that subject (7). A similar individual peculiarity can be seen in Fig. 2 with regard to the insulin areas. Subject II and Subject IV were each taking a diet containing 50 g. of carbohydrate yet their curves are not identical; Subject I was receiving a diet containing 100 g. of carbohydrate, Subject III one containing 215 g. yet their curves lie close together. Nevertheless the similarity of the curves suggests that the factors of diet and individual peculiarity are but

superimposed upon a constant relationship and that if they could be eliminated this relationship would be revealed. This elimination is performed as follows. The insulin area corresponding to 5 units is read off from the curve for each individual. This value is then divided into the insulin area for each dose of insulin and this quotient plotted against the insulin dosage. The result is shown in Fig. 3, and it will be seen that by this means the points for

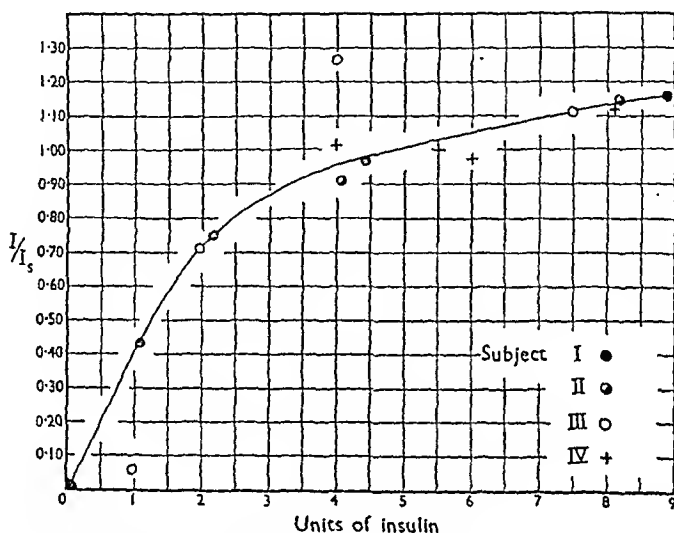


Fig. 3. Curve expressing the relationship for healthy subjects in general between the dose of insulin and the degree to which it suppresses alimentary hyperglycæmia. The curve for each individual (Fig. 2) is influenced in height by the composition of the diet and unknown factors arising from personal idiosyncrasy. These individual influences are eliminated by reading off from each curve in Fig. 2 the area corresponding to 5 units of insulin (I_5) and dividing this into each of the areas observed (I) for the different doses. The curve relating this ratio I/I_5 to the dose of insulin is shown above.

the different subjects are brought into line and a curve obtained expressing the relationship between the insulin area and the insulin dosage which is of general application for normal subjects. Given the insulin area produced by 5 units of insulin in any particular subject, the insulin areas which would be produced by other doses of insulin can be calculated by means of this curve.

Scott and Dotti (9) have claimed that a linear relationship exists between the effects of insulin on the blood sugar level and the logarithm of the dose of insulin given. For the smaller doses a linear logarithmic relationship can be established for our results, but for doses of insulin greater than 4 units the insulin area does not increase sufficiently rapidly to conform to this linear graph. In our opinion, when Scott and Dotti's figures are examined the logarithmic relationship will be seen to hold only when small doses of insulin are given and the same lack of conformity as that shown by our figures

exists for their larger doses. The fact that in our results a logarithmic proportion exists between insulin dose and insulin effect when small doses are used but fails to satisfy the figures when larger doses are used suggests that when large doses of insulin are injected some interfering factor comes into play as so to reduce insulin effect. Such a factor may well be the compensatory outpouring of blood sugar which is known to be provoked by the hypoglycæmia consequent on the larger doses of insulin. In view of this interference it appears unprofitable to pursue farther this mathematical analysis of our curve.

The above information was sought, not for its own sake, but because it was hoped that it would open the way to the elucidation of a more difficult problem, namely the effect of variations in the degree of alimentary hyperglycæmia, all other factors remaining constant, upon the action of a constant dose of insulin. Until this relationship had been clarified it was obviously impossible to say that the effect of a standard dose of insulin upon the high blood sugar curve of a diabetic was less, equal to or greater than the effect of the same dose upon the relatively low sugar curve of a normal person. It was known that in the fasting state the higher the blood sugar level the greater the depression produced by a standard dose of insulin and the important work of Scott and Dotti (9) in this field has clarified this relationship between the degree of depression and the initial level of the blood sugar. It seemed probable that a similar relationship would hold between the degree to which a constant dose of insulin suppressed alimentary hyperglycæmia and the degree of alimentary hyperglycæmia produced by the same dose of glucose, when no insulin was given.

The most obvious approach to the problem was to devise methods by which the height of the blood glucose tolerance curve could be varied whilst the dose of insulin and the other experimental conditions were kept constant. This is, as far as we are aware, impossible. It was shown by Hansen (2) that increasing the test dose of glucose did not alter the degree of hyperglycæmia but only its duration and it has also been shown by Cori (1) and by Verzár (10) that as long as there is any glucose in the intestine absorption proceeds at a constant rate. It is, therefore, probable that any lowering of the degree of hyperglycæmia produced by decreasing the test dose of glucose is due to earlier cessation of absorption. Clearly the effect of a constant dose of insulin upon a hyperglycæmia when absorption has ceased will not be quantitatively comparable to the effect of the same dose when absorption is still proceeding. Similarly it is not allowable to vary the degree of hyperglycæmia by altering the carbohydrate content of the diet, or by reinforcing the alimentary hyperglycæmia by injecting adrenalin or pituitrin, for these agents of themselves alter the efficiency of insulin. The direct approach to the problem of the relationship between the degree of alimentary hyperglycæmia and the effect of insulin in suppressing it being thus impracticable we were compelled to attack the problem indirectly through the data presented in this section. This involved making the

assumption that the total effect of a given dose of insulin was the arithmetical sum of the effect of its component units.

The circulation is as follows. If x units depress a glucose tolerance area G to a residual area R , and $2x$ units depress the glucose tolerance area G to a residual area R_2 then the effect of x units acting on a tolerance area of

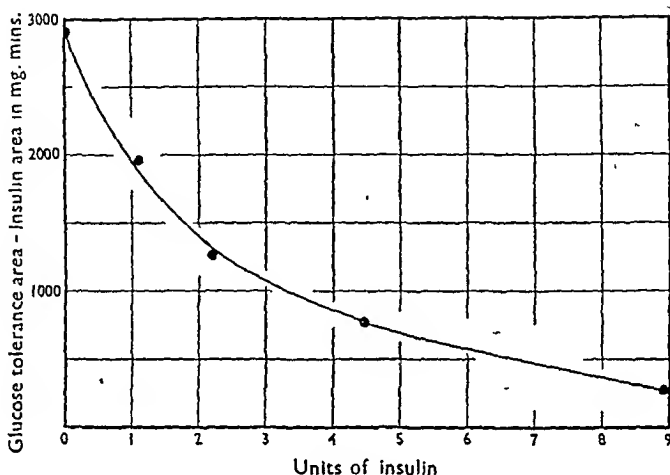


Fig. 4. Curve showing the relationship in Subject I between the dose of insulin and the residual alimentary hyperglycemia (glucose tolerance area-insulin area) under constant conditions of diet and environment. From this curve the effect of the degree of alimentary hyperglycemia upon the action of a standard dose of insulin can be read off. Example: 2 units of insulin acting on a glucose tolerance area of 2920 mg. min. reduces this area to a residuum of 1380 mg. min.. The effect of 2 units of insulin acting on a hyperglycemia of 2920 mg. min. is therefore 1540 mg. min.. At a hyperglycemia of 1380 mg. min. 2 units of insulin result in a residual area of 850 mg. min. and therefore its effect at a hyperglycemia of 1380 mg. min. is only 530 mg. min..

TABLE II.

Table showing the insulin area and the I/G ratio corresponding to different degrees of alimentary hyperglycemia, as measured by the glucose tolerance area, when dietetic and environmental factors are constant.

Subject I.			Subject II.			Subject III.			Subject IV.		
Glucose tol. area (G)	Insulin area (I)	I/G	Glucose tol. area (G)	Insulin area (I)	I/G	Glucose tol. area (G)	Insulin area (I)	I/G	Glucose tol. area (G)	Insulin area (I)	I/G
2920	1540	0.55	3920	1960	0.50	2520	1480	0.59	5740	2360	0.41
1380	530	0.38	1960	960	0.49	1040	540	0.52	3380	1220	0.36
850	330	0.39	1000	450	0.45	500	270	0.54	2160	660	0.31
520	190	0.38	550	270	0.49	230	130	0.56	1500	280	0.19

Examples of the method of calculating these figures are given in the text and in the legend to Fig. 4.

R_1 would be $R_1 - R_2$ mg. mins. This relationship is expressed in a curve in which the observed residual area R is charted against the dose of insulin producing it (Fig. 4). From such a curve the effect of any dose of insulin acting at any blood sugar level within the range of the curve can be read off, as shown in the example given in the legend of Fig. 4. Curves of this type were charted for each of the four individuals and from them the effect of a constant dose of insulin on different glucose tolerance areas was read off. The results are given in Table II, and it can be seen that decrease in the height of the glucose tolerance curve is associated with a decrease in the absolute effect of the standard dose of insulin. The relationship between glucose tolerance and insulin effect can be conveniently expressed by the ratio between the insulin area, I , and the glucose tolerance area, G , on which it acts. It can be seen from Table II that, as the glucose tolerance area decreases, the I/G ratio either remains approximately constant or decreases. Our results do not permit us to decide which of these two types of change in the I/G ratio is the correct one but for the purpose of interpreting the results in the next section of this paper it suffices to establish the point that as the glucose tolerance area decreases the I/G ratio *does not increase*. This means that, under constant conditions of diet and environment, the insulin area, due to a constant dose of insulin, decreases at a rate proportional to or proportionately faster than the diminution in the glucose tolerance area and that it does *not* diminish at a rate proportionately slower than the glucose tolerance area. We suggest, therefore, the following conclusion, *the effect of insulin in suppressing alimentary hyperglycæmia varies with the degree of hyperglycæmia on which it acts*.

II. *The effect of a constant dose of insulin upon alimentary hyperglycæmia when the composition of the diet is varied.* The plan of the experiments for each of the four subjects was as follows. After a period on each of the different diets a blood glucose tolerance test and two days later an insulin glucose test were carried out. The dose of glucose, both for the glucose tolerance and the insulin glucose test, was 30 g. per sq. m. of body surface; the dose of insulin was 5 units per sq. m. of body surface. All diets had the same caloric value of 2680 calories and two series, each of four diets, were used. Both series commenced with the diet containing 50 g. of carbohydrate and ended with the diet containing 500 g. carbohydrate. The composition of the two series of diets is shown in Table III.

The quantitative relationship between change in glucose tolerance and change in composition of the diet has been established previously and deviation from this relationship in a particular case can be taken as an indication that some disturbing factor has intruded. Such factors are sepsis, emotion and breaking of diet. Of the four subjects used for these experiments Subjects I and IV remained in a satisfactory state throughout the test period; Subject III showed signs of a mild chronic respiratory infection during the second part of his employment; Subject IV was emotionally labile and was the least satisfactory of the four. It can be seen from Table

TABLE III.

Diets used for different subjects.

Subjects I and III.				Subjects II and IV.			
Carb.	Prot.	Fat.	Calories.	Carb.	Prot.	Fat.	Calories.
50	80	240	2,680	50	80	200	2,680
100	80	218	2,680	165	80	189	2,680
215	80	166	2,680	280	80	138	2,680
335	80	113	2,680	390	80	89	2,680
500	80	40	2,680	500	80	40	2,680

IV that as the diet is changed the percentage improvement in glucose tolerance of Subjects I and IV agrees with the results previously reported (7) whilst certain curves from the other two subjects do not conform with these results. In these experiments, therefore, we place more reliance upon the results from Subjects I and IV than on those from Subjects II and III.

The effect of increasing the amount of carbohydrate in the diet both on the glucose tolerance curve and on the insulin-glucose curve is shown in Fig. 5 and Table V. In Fig. 5 is shown the effect in one subject of changing

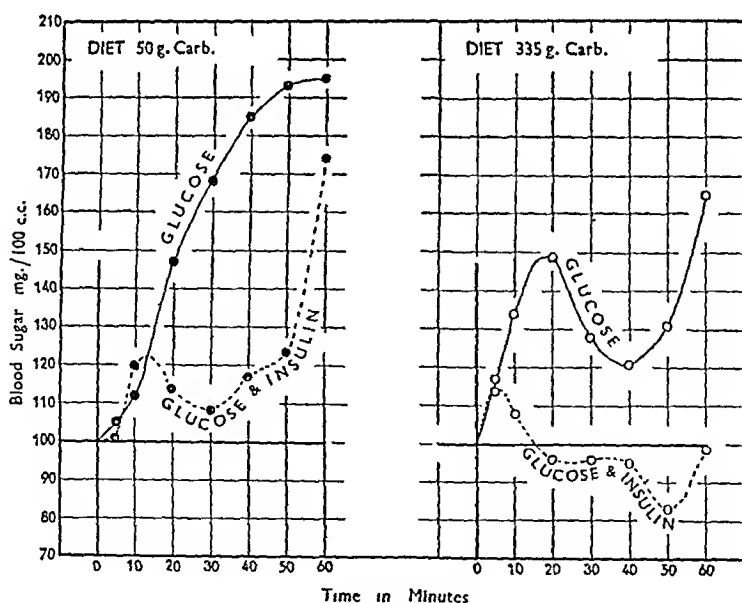


Fig. 5. The effect of increasing the carbohydrate content of the diet from 50 g. (dices) to 335 g. (circles) upon the glucoso tolerance curve (solid line) and the insulin-glucoso curve (broken line). Both these curves decrease in height but the area included between them remains practically constant.

the diet from one containing 50 g. of carbohydrate to one containing 335 g. of carbohydrate and it can be seen that both the glucose tolerance and insulin-glucose curves decrease in height as the dietary carbohydrate is increased. In Table V the effect for each of the four subjects of each particular diet on the glucose tolerance area and insulin area is recorded. From a study of these areas various points emerge.

As the carbohydrate in the diet is increased the glucose tolerance area decreases but the insulin area either does not change or changes to a far less extent. This point is well brought out by the figures obtained on the most satisfactory subject, No. I. Here it will be seen that increasing the dietary carbohydrate from 50 g. to 335 g., which causes an improvement in the glucose tolerance area to 60 mins of 101%, causes practically no change in the insulin area. On increasing the dietary carbohydrate to 500 g. however the insulin area decreases to 1820 mg. mins although the glucose tolerance area increases to 118%. The last phenomenon can be seen in the figures for each of the other three subjects; in each case the insulin area on the diet containing 500 g. of carbohydrate is smaller than the area in the

TABLE IV.

Table showing the effect of the carbohydrate content of the diet upon the glucose tolerance of each of the four subjects used in this investigation.

Carbo- hydrate content of diet gms.	Subject I.		Subject III.		Carbo- hydrate content of diet gms.	Subject II.		Subject IV.	
	Glucose tol. area.	% Improve- ment.	Glucose tol. area.	% Improve- ment.		Glucose tol. area.	% Improve- ment.	Glucose tol. area.	% Improve- ment.
50	8,900	0	14,000	0	50	7,900	0	9,100	0
100	6,650	60	10,450	55	165	4,740	91	4,650	78
215	6,150	73	7,560	98	280	7,700	5.8	3,470	98
335	6,270	73	10,150	60	390	5,690	64	3,190	104
500	4,480	118	6,400	118	500	3,840	118	2,360	118

In a previous publication (7) increasing the dietary carbohydrate from 50 g. to 500 g. was taken as causing an improvement in glucose tolerance of 118%. The improvement in the present experiments have been expressed on the same basis. All curves on a particular subject were terminated at the shortest time taken of any curve in the series to return to normal (Subject I, 138 min.; II, 129 min.; III, 106 min.; IV, 94 min.). It will be seen that the improvement in Subjects I and IV conformed to that previously established, whilst Subjects II and III showed discrepancies.

previous diet containing less carbohydrate. In our opinion this last phenomenon is an artefact. When a normal subject receives a diet containing 500 g. of carbohydrate the degree of hyperglycæmia upon which insulin acts is so small and the sensitivity to insulin so great that shortly after the insulin is injected the blood sugar falls to levels sufficiently low to provoke a compensatory outpouring of sugar from the liver into the blood. This

inflow of sugar is added to that already entering the blood stream from the gut, the insulin glucose curve consequently rises too high and the insulin area is correspondingly diminished. For these reasons we attach little importance to the insulin areas on the diet containing 500 g. of carbohydrate.

TABLE V.

Table showing the effect, in each of the four subjects, of the carbohydrate content of the diet upon the glucose tolerance area and upon the insulin-glucose area measured to 60 min.

Carbo- hydrate content of diet in g.	Subject I.		Subject III.		Carbo- hydrate content of diet in g.	Subject II.		Subject IV.	
	Glucose tol. area.	Insulin area.	Glucose tol. area.	Insulin area.		Glucose tol. area.	Insulin area.	Glucose tol. area.	Insulin area.
50	3,940	2,610	5,510	4,750	50	{4,480 {3,910	{3,490 {2,990	5,740	4,510
100	{3,130 {2,920	{2,940 {2,640	3,430	4,060	165	2,900	2,720	3,168	3,630
215	2,680	2,640	2,520	2,360	280	3,220	3,410	2,430	2,810
335	2,160	2,360	3,770	4,650	390	4,020	4,150	2,380	3,340
500	1,870	1,820	3,040	2,500	500	1,960	2,460	1,940	2,390

The bracketted figures are duplicate estimations.

The general significance of these results (Table V) appears to be that, although increase of dietary carbohydrate improves glucose tolerance (decreases the degree of hyperglycæmia), it has relatively little effect upon the insulin area which changes only slightly or remains about the same value; that is, under these conditions the insulin area is more than proportional to the degree of hyperglycæmia upon which insulin acts. This is in contrast to the constant or decreasing relationship between the insulin area and the degree of hyperglycæmia which, in the previous section, was shown to exist when the composition of the diet was kept constant. This contrast is well brought out by comparing the values of the ratio insulin area I, to the glucose tolerance area, G, when the dietary carbohydrate is varied with the ratio when the diet is constant. When the dietary carbohydrate is increased the I/G ratio increases also (Table VI); when the dietary carbohydrate is kept constant the I/G ratio remains relatively constant or decreases (Table II). This comparison indicates that when the dietary carbohydrate is changed the degree of hyperglycæmia conditioned by the change is not the only factor which determines the extent to which insulin acts, for if this were so the I/G ratio would have remained constant or have decreased. Some other factor facilitating the action of insulin and increasing its effect comes into play so that, although the degree of hyperglycæmia diminishes and the insulin is thus forced to act at a disadvantage, the efficiency with which the insulin removes the blood sugar remains unimpaired. This point

is well brought out by the figures in Table VII. Taking the ratio that exists between the insulin area and the glucose tolerance area on the diet containing 50 g. of carbohydrate as a basis, we have calculated the insulin area which would correspond to the glucose tolerance area produced by each diet were the degree of hyperglycæmia the only factor determining the size of the insulin area, that is the I/G ratio had remained constant. On comparing the insulin areas thus calculated with the insulin areas actually observed when the different diets were being given, it will be seen that the observed

TABLE VI.

Table showing for each of the four subjects the effect of the carbohydrate content of the diet upon the ratio insulin area (I) glucose tolerance area (G).

Carbo- hydrate content of diet in g.	I/G		Carbo- hydrate content of diet in g.	I/G	
	Subject I.	Subject III.		Subject II.	Subject IV.
50	0.66	0.86	50	{ 0.78 0.76	0.79
100	{ 0.94 0.91	1.18	165	0.94	1.14
215	0.98	0.94	280	1.06	1.16
335	1.09	1.23	390	1.04	1.40
500	(0.98)	(0.83)	500	(1.25)	(1.23)

TABLE VII.

Table comparing the observed insulin area with calculated insulin area which would be expected if the only factor determining the insulin area produced by a constant dose of insulin, were the degree of alimentary hyperglycæmia upon which the insulin acted.

Carbo- hydrate content of diet in g.	Subject I.		Subject III.		Carbo- hydrate content of diet in g.	Subject II.		Subject IV.	
	Insulin area.		Insulin area.			Insulin area.		Insulin area.	
	Calcu- lated.	Ob- served.	Calcu- lated.	Ob- served.		Calcu- lated.	Ob- served.	Calcu- lated.	Ob- served.
50	2,610	2,610	4,750	4,750	50	{ 3,490 3,050	{ 3,490 2,990	4,510	4,510
100	{ 2,070 1,930	{ 2,940 2,640	2,950	4,060	165	2,260	2,720	2,500	3,630
215	1,770	2,640	2,160	2,360	280	2,510	3,410	1,920	2,810
335	1,420	2,360	3,240	4,650	390	3,140	4,150	1,880	3,340
500	1,230	1,820	2,600	2,500	500	1,530	2,460	1,530	2,390

areas are, with two minor exceptions, greater than the calculated areas. In other words some factor has come into play to offset the effect of diminishing hyperglycemia in reducing the absolute effect of insulin action.

As the only variable in this present series of experiments is the amount of dietary carbohydrate it would seem that the appearance of this factor is conditioned by the carbohydrate content of the diet and that further a relationship should exist between the amount of dietary carbohydrate and the influence exerted by this factor. Such a relationship can be demonstrated by means of the I/G ratio. Disregarding the figures for the diet containing 500 g. of carbohydrate, for the reasons given above, it will be seen from Table VI that with each increase of the carbohydrate content of the diet there is also an increase in the value of the I/G ratio. This point is further illustrated in Fig. 6 where it is shown that change in the I/G ratio, expressed as a percentage of the total change between the first and fourth diet of the series, is correlated with change in dietary carbohydrate.

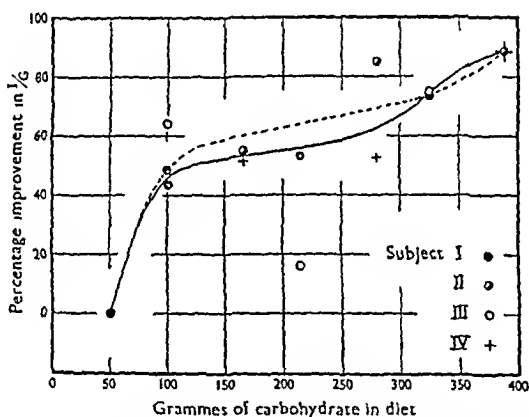


Fig. 6. Curve (solid line) correlating the variation in the carbohydrate content of the diet and the corresponding change in the ratio between the insulin area (I) and the glucose area (G) expressed as a percentage of the total change. For comparison the curve (broken line) previously found to correlate variation in the carbohydrate content of the diet with the percentage change in insulin sensitivity is given, and it will be seen that the two curves are practically the same. The figures have been expressed in the same basis as was chosen previously (7) namely that a change from 50 g. to 390 g. of carbohydrate causes an 88% improvement in insulin sensitivity.

In a previous series of papers (5) (6) (7) the effect of increasing the dietary carbohydrate upon the efficiency with which insulin acts upon the blood sugar in the fasting state has been demonstrated and it has been shown that the increasing sensitivity to insulin thus induced is correlated with a commensurate improvement in glucose tolerance. Table V and VI show that change in the I/G ratio is similarly correlated with change in glucose tolerance and reference to Fig. 6 shows further that the curve correlating change in the I/G ratio with change in the dietary carbohydrate and the

curve previously established (7) as correlating change in the insulin sensitivity with change in dietary carbohydrate, are practically identical. We conclude from these similarities that the mechanism which brings about increasing insulin sensitivity as the diet changes is the same as that which brings about increase in the I/G ratio and that, therefore, *in normal subjects the change in the I/G ratio is a measure of the change in insulin sensitivity.*

The object of this paper was not merely to offer alternative evidence of conclusions already arrived at, but to manufacture a new weapon for attacking the problem of insulin sensitivity in diabetics. The fact that the fasting blood sugar level of different diabetics varies widely nullified our efforts to compare their insulin sensitivity by measuring the effect of insulin upon their blood sugar level in the fasting state. Thus the application to diabetics of the previous work on insulin sensitivity in normal subjects was prevented. By using the I/G ratio this difficulty was avoided for the I/G ratio is a measure of the effect of insulin on alimentary hyperglycæmia regardless of the blood sugar level. The results of this method applied to diabetic patients will be published shortly. In this connection attention may be drawn to an important corollary of the above conclusions. If it is found that under one set of conditions there is a high degree of alimentary hyperglycæmia and under a second set a low degree and yet under both conditions the effect of a standard dose of insulin, as measured by the insulin area, is the same, then the insulin sensitivity under the second set of conditions is greater than under the first set. And by means of the I/G ratio this masked change in sensitivity can be revealed and expressed quantitatively.

The results presented in this paper provide another example of the close correlation existing between the glucose tolerance and the sensitivity to insulin. It is here shown that when the degree of hyperglycæmia is raised by restriction of dietary carbohydrate the effect of the increased hyperglycæmia in improving the efficiency with which insulin acts is nullified by a decrease in insulin sensitivity. The inference suggested by this observation is that when conditions impair the sensitivity of the body to insulin a compensatory increase in the degree of alimentary hyperglycæmia is brought about in an effort to maintain the efficiency of the endogenous insulin action at a constant level. The occurrence of a relationship between the level of the blood sugar and insulin action which is essentially similar to the Mass Action Law has been suggested previously (4). A considerable amount of evidence is now available in support of this suggestion and in a forthcoming paper it will be collected and discussed.

SUMMARY.

1. The effect of insulin in suppressing alimentary hyperglycæmia has been investigated, under varying conditions in four healthy human subjects.

2. (a) The relationship between insulin dosage and the degree to which it suppresses alimentary hyperglycæmia has been investigated and it has been

shown that the results can be expressed by a curve of similar form in each individual. By correcting each result for individual peculiarity and the effect of diet the separate individual curves can be brought to lie on one common curve which expresses the general relationship between these two variables.

(b) From the same results the effect of a constant dose of insulin acting upon varying degrees of alimentary hyperglycæmia, when all other conditions are kept constant, has been calculated. The results indicate that under such conditions the effect of a standard dose of insulin varies with the degree of alimentary hyperglycæmia on which it acts.

3. (a) The effect of varying the composition of the diet upon the efficiency with which a standard dose of insulin suppresses alimentary hyperglycæmia has been investigated, and it has been shown that whilst increase of dietary carbohydrate improves glucose tolerance it apparently causes little if any improvement in the extent to which insulin suppresses the hyperglycæmia. This apparent constancy of insulin effect may be explained on the grounds that, as the dietary carbohydrate is increased, the body becomes more susceptible to insulin and this increased susceptibility nullifies the influence of the diminishing hyperglycæmia, conditioned by the dietary change, in reducing insulin action.

(b) Change in susceptibility, thus masked, is revealed by change in the ratio of suppression of hyperglycæmia (insulin area, I) to degree of hyperglycæmia (glucose tolerance area, G). The relationship between change in the carbohydrate constant of the diet and change in this I/G ratio is expressed by a curve similar to that which expresses the relationship between change in dietary carbohydrate and change in insulin sensitivity, as measured by the effect of insulin in the fasting state. It is concluded that increase in insulin sensitivity potentiates to a similar extent the action of insulin both in lowering the fasting blood sugar and in suppressing alimentary hyperglycæmia, and that the I/G ratio is a convenient method of measuring the degree of insulin sensitivity in the presence of alimentary hyperglycæmia.

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HYPERPNOEA IN HEART FAILURE.

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Introduction.

DYSPPNOEA in heart failure is a complex problem, the solution of which still remains elusive. In his excellent review Christie (6) sums up by emphasising the importance of pulmonary congestion. There still exists, however, some confusion as to the exact part played by pulmonary congestion in producing the picture of dyspnoea and it seems that this is due to a failure clearly to separate the phenomena of dyspnoea from those of hyperpnoea. Dyspnoea is difficult breathing. Hyperpnoea is increased ventilation. A patient may be hyperpnoeic but not dyspnoeic, as in diabetic acidosis. The classical researches of Peabody and Wentworth (22) showed that when the vital capacity is diminished, the cardiac patient becomes conscious of dyspnoea at a much lower volume of pulmonary ventilation than does a normal subject. None the less the stimulus of hyperventilation has to be present to produce dyspnoea. In cardiac patients who are dyspnoeic at rest hyperventilation is always present as well.

The cause of the hyperpnoea at rest still seems to be in some doubt. Fraser (9) ascribed it to diminished blood flow through the brain. Modern work by the acetylene method on cardiac output has revealed, however, that, until the late stages of the disease at least, the cardiac output may not be much depressed below the normal value. In view of this, doubt was cast on the conception that a slow circulation rate was the cause of hyperpnoea. The more direct observations of Harrison (13) and his co-workers on the gaseous content of internal jugular venous blood also failed to reveal evidence of diminished cerebral blood flow in patients suffering from cardiac dyspnoea, and they were thus compelled to seek other causes. They indicated that hyperpnoea might result from reflexes initiated in the engorged veins and the congested lungs. The hyperpnoea was thus considered to be reflex. There is a difficulty in accepting this reflex hypothesis in that it implies an alteration in chemical sensitivity of the respiratory centres. The pulmonary ventilation is essentially regulated by the pH and CO₂ tension in the respiratory centre. "Normally nervous

* Johnston and Lawrence, Research Fellow of the Royal Society, working with grants from the Medical Research Council and Royal Society.

reflexes modify chiefly the *rate* and change the depth of breathing inversely " (Wiggers (24)). If respiration is stimulated by nervous reflexes, CO_2 is washed out of the blood, leading to depression of respiratory activity so that the final result is no significant change in the total ventilation. The only exception is the reflex hyperpnœa resulting from the effects of low oxygen tension on the cells of the carotid body (7). This effect, however, is probably essentially a chemical control acting through what may be considered as an outlying atavistic portion of the respiratory centre. Gesell and Moyer (10) suggested the possibility that vagal reflexes may modify the response of the respiratory centre to CO_2 and pH, but there is no satisfactory evidence that reflexes produce a continuously maintained change in the total ventilation such as we observe in the hyperpnœa of heart failure.

In view of these difficulties it was decided to survey a considerable number of data in which cardiac output, pulmonary ventilation and vital capacity have been measured simultaneously in order to establish the relations existing between them.

Methods.

Data are presented from 22 patients, 21 adults and 1 adolescent, all but two being males. They had heart disease of different degrees, and were graded I, IIa, IIb, and III according to its severity.*

The arterio-venous oxygen difference was measured by the acetylene method, and pulmonary ventilation was measured by collection in a Douglas bag, the expired air being analysed in a van Slyke or Haldane apparatus. Vital capacity was measured by an ordinary spirometer.

Normal standards of pulmonary ventilation were taken from (1) 28 personal observations on normal subjects at rest, and from (2) data available in this laboratory from recent basal metabolism determinations (Douglas bag method) on 48 patients suffering from conditions not known to affect respiratory function, such as rheumatoid arthritis, obesity, amenorrhœa, simple goitre, varicocele, etc. Patients in the second group had pulse rates under 80, and the basal metabolic rate fell between -10% and $+15\%$. To simplify the calculations, the figures for pulmonary ventilation and oxygen consumption were uncorrected for temperature and pressure, the oxygen consumption being calculated from the percentage oxygen utilisation \times the pulmonary ventilation as measured from the volume per minute of expired air at room temperature.

Normal standards for pulmonary ventilation.

Absolute measurements of ventilation lose much of their value if the rate of O_2 consumption is not taken into account, for it is at least an approx-

* In Grade I there is no complaint of dyspnœa : in Grade IIa dyspnœa is present on moderate exertion such as climbing stairs. At stage IIb dyspnœa is present with such ordinary activity as walking on the level. At stage III breathlessness is present at rest. The Roman numeral used in each case number indicates its classification.

imate physiological law that the pulmonary ventilation increases in proportion to the metabolic rate. For purposes of measurement the ventilation equivalent was first suggested by Anthony (1). It is the volume of air respired per 100 c.c. O_2 taken up by the body. This ventilation equivalent for O_2 was adopted as one of the important measures of respiratory efficiency by Moncrieff (21). In spite of the apparent value of such a measurement it has not met with general acceptance. Hurtado and Boller (14) found from 15 observations that the ventilation equivalent had an average value of 2.6 with variations between 1.47 and 4.04. The standard deviation was 0.73. If their findings are substantiated, then it is obvious that this ventilation equivalent has too wide a scatter to be of much practical use. Christie and McIntosh (5) bring forward a more theoretical objection to the ventilation equivalent. They point out that since

$$\frac{\text{minute vol. respired} \times 100}{O_2 \text{ cons./min.}} = \frac{\text{minute vol. respired} \times 100}{\text{min. vol. respired} \times O_2\% \text{ absorbed}}$$

$$= \frac{100}{O_2\% \text{ absorbed}}$$

the minute volume respired need not be measured in calculating the equivalent. While this statement is undoubtedly correct, the ventilation equivalent still preserves its meaning, namely, the volume of air respired per 100 c.c. O_2 consumed. It has already been shown (16) that the ventilation equivalent undergoes a change which can be correlated with postural alterations in circulatory minute volume. Ventilation equivalents, however, cannot be satisfactorily compared unless the respiratory quotient is known to remain reasonably constant. Marais and McMichael (20) suggested the use of a ventilation equivalent for CO_2 on theoretical grounds, stating that this equivalent would be more likely to be constant than that for oxygen in the presence of a varying respiratory quotient.

TABLE I.
Ventilation equivalents (V.E.) in 76 normal individuals.

V.E. O_2	Frequency.	V.E. CO_2	Frequency.
1.6—1.89	2	2.2—	8
1.9—	16	2.5—	16
2.2—	26	2.8—	16
2.5—	16	3.1—	19
2.8—	8	3.4—	12
3.1—	7	3.7—	3
3.4—	1	4.0—	2
Mean 2.49 s.d. .40 Coefficient of variation 16%		Mean 3.06 s.d. .44 Coefficient of variation 14%	

Table 1 shows that the average ventilation equivalent for oxygen is 2.49 with a standard deviation of 0.40 while the same ratio for CO_2 is 3.06, the standard deviation being 0.44. There is thus a similar range for normal values of both these ratios, and the scatter is much less than that observed by Hurtado and Boller.

The ventilation equivalent may vary in any given individual, however, with fortuitous changes in the respiratory quotient. It is essential to remember the Haldane concept that respiration is delicately adapted to regulate the CO_2 pressure in the blood. For the same rate of oxygen consumption a varying amount of CO_2 may have to be eliminated as the respiratory quotient changes. Apart from this, however, in short periods of Douglas-bag collection over-breathing or under-breathing may take place. As the subject over-breathes for example, the oxygen consumption rate remains essentially unaltered and thus the ventilation equivalent for oxygen rises in value; the volume of CO_2 expired increases, however, with over-ventilation, and thus great fluctuations in the ventilation equivalent for CO_2 are prevented. In one subject, who may be taken as typical (Table II)

TABLE II.

Variations in ventilation equivalents (V.E.) with alterations in respiratory quotient (R.Q.) in one individual.

V.E. O_2	R.Q.	V.E. CO_2
1.84	.74	2.48
1.95	.77	2.53
2.12	.78	2.71
1.93	.79	2.44
2.24	.79	2.83
2.07	.79	2.62
1.98	.79	2.51
1.92	.80	2.40
1.87	.81	2.31
2.24	.81	2.76
2.21	.82	2.69
2.04	.82	2.48
2.17	.83	2.61
2.19	.84	2.61
2.50	.85	2.94
2.11	.86	2.45
2.38	.90	2.54
2.38	.90	2.64
2.28	.92	2.47
2.57	.94	2.71
2.77	.98	2.83
3.11	1.09	2.86
Mean = 2.22 s.d. = 0.30 Coefficient of variation = 13.5%		Mean = 2.61 s.d. = 0.16 Coefficient of variation = 6%

the ventilation equivalent for oxygen gave mean values of 2.22 (s.d. 0.30). Fig. 1 shows that the ventilation equivalent for O_2 varied with the respiratory quotient, while the ventilation equivalent for CO_2 was less variable having

a mean value of 2.61 (s.d. 0.16). Thus so far as the individual subject is concerned the ventilation equivalent for CO_2 has a distinct advantage in being less subject to accidental changes, and it has therefore been used in the course of this work.

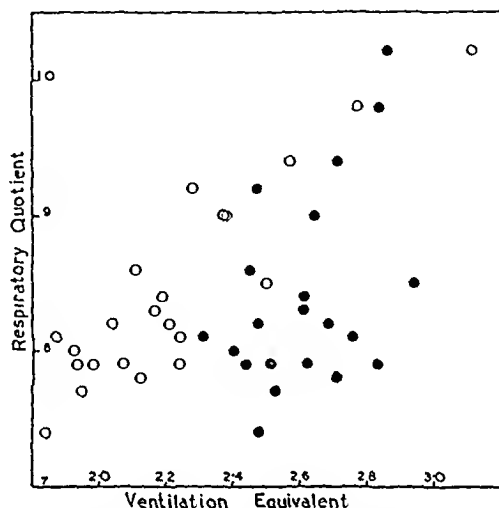


Fig. 1. Ventilation equivalent for oxygen (circles) and ventilation equivalent for CO_2 (dots) plotted against the respiratory quotient: data from one individual. The ventilation equivalent for oxygen varies with the respiratory quotient, while the ventilation equivalent for CO_2 is less liable to variation.

The accuracy of cardiac output determination.

In a previous paper (16) it was pointed out that there were three main sources of error in the determination of the arterio-venous oxygen difference by the acetylene method. These were (1) incomplete mixing in the lung-bag system, (2) recirculation of acetylene-containing blood, and (3) analytical errors. A technique was described for reducing the third source of error.

From 100 consecutive determinations made by the writer the probable error of the acetylene method may be evaluated. The estimations of the arterio-venous oxygen difference are made from samples taken at the 5th, 6th and 8th expirations. Errors due to incomplete mixing are most liable to occur in the first sample while errors due to recirculation would tend to occur in the third sample. In the third sample, however, the mixing error may be regarded as eliminated. In calculating the answers the oxygen and acetylene differences are taken between the first and third, and between the second and third samples. Since the time between the first and second samples is small the percentage differences tend to be small, and small analytical or sampling errors therefore render determinations from these two samples less reliable. Any mixing error present in the first sample is

usually rendered obvious by a gross difference between the two results. For this reason results differing by more than 10% from their mean value are invariably discarded, although their frequency (15/100) is indicated in Table III, in which the results obtained in 100 consecutive cardiac output determinations are summarised. The standard error of the mean of two determinations is 4.5%.

TABLE III.

Error of the acetylene method in 100 consecutive determinations.

The arterio-venous oxygen difference calculated from samples 2 and 3 is expressed as a percentage deviation (irrespective of sign) from the estimation made from samples 1 and 3.

Percentage difference.	Frequency.	Sum of squares.	σ^2
0-5	32		
5-10	29		
10-15	15		
15-20	9	6940	82.5
20-30	6		
30-40	5		
40-50	3		
50-60	1	25920	259.2

For the whole distribution—Standard deviation of a single determination from the mean value ($\sigma/\sqrt{2}$) = 11.4%.

If estimations differing by over 20% (10% from mean value) are discarded as having a systematic "mixing" error—

Standard error of a single determination ($\sigma/\sqrt{2}$) = 6.4%.

„ „ „ average of two determinations = 4.5%.

If recirculation were an important source of error we should expect that the result calculated from the second and third samples would give a lower rate of acetylene disappearance, and therefore a *higher* value for the arterio-venous oxygen difference than the result calculated from the first and third samples. In surveying these 85 acceptable determinations 39 give higher values (mean = +7.67%) for the arterio-venous oxygen difference while 46 give lower values (mean = -8.9%). There is thus no statistical evidence that recirculation is an important source of error. This agrees with the observations of Christensen and Trolle (4) who found that blood returning in the veins contained much less acetylene than might have been expected owing to diffusion into the tissues. Gladstone (11) over-emphasised the possibility of error from recirculation.

The relations of cardiac output, vital capacity and ventilation equivalent in heart disease.

A series of 61 of these measurements is shown in Table IV. They were obtained in 21 cases of heart failure at various stages. If increased ventilation results from pulmonary congestion, there should be an obvious correlation when the ventilation equivalent is plotted against the vital capacity, since the latter is lowered in proportion to the degree of congestion.

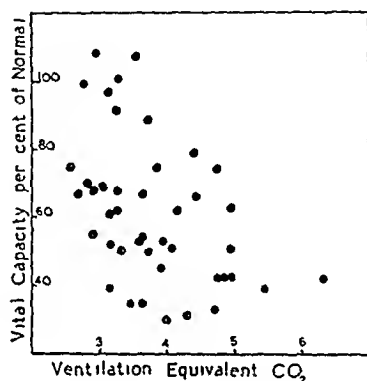


Fig. 2. Vital Capacity is plotted as a percentage of the expected value. The correlation between ventilation and depression of vital capacity ($r = -0.39$) is not striking.

In Fig. 2 the vital capacities are plotted as percentages of the expected normal values, from the formula

$$\text{Vital capacity} = 0.169 (\text{height in inches}) - 7.34.$$

The standard error of the predicted value is ± 0.39 litre (2). The coefficient of correlation between ventilation equivalent and vital capacity ($r = -0.39$, s.e. ± 0.13) is too low to allow the acceptance of the hypothesis that pulmonary congestion is the principal cause of cardiac hyperpnoea.

In individual cases the relationship between cardiac output and pulmonary ventilation may not be obvious. This is readily understandable when we take into account the error to which each measurement is liable, and the relatively slight changes which may occur in cardiac output when a patient passes from one stage of the disease to another. In Case III 3, however, thirteen observations were made as the patient passed from Grade III failure towards normal on digitalis therapy. These are plotted in Fig. 3, and they show a definite curvilinear arrangement, which is also obvious when the whole series is surveyed collectively (Fig. 4). It is quite apparent without applying further corrections for size of the individuals that the hyperpnoea of heart failure is much more closely correlated with cardiac output than with vital capacity.

TABLE IV.

Ventilation equivalent (V.E.), arterio-venous oxygen difference (A.V.O₂), actual cardiac output in litres per min. (Abs.C.O.), and vital capacity (V.C.), in cardiac disease.

Case and height (ins.)	Diagnosis.	V.E. CO ₂	A.V. O ₂	Abs. C.O.	V.C.	Log. V.E.	Log. C.O.
*I 2 (67½)	Atheromatous coronary dis.	3·84 3·27	82 67	4·54 4·96		·584 ·515	·657 ·695
*I 3 (68)	Congenital aortic stenosis.	2·78	64	5·91	4·1	·444	·772
*I 4 (68)	Coronary thrombosis.	2·96	68	5·18	4·5	·471	·714
I 7 (61)	Patent ductus.	3·13	64	3·41	2·9	·496	·533
*IIa3 (64½)	Rheumatic, mitral and aortic dis.	3·58 4·0	84 96	3·84 3·27		·554 ·602	·584 ·515
*IIa4 (72)	Rheum. mitral dis. Fibrillation.	4·45 3·41 3·11 3·71 4·95 4·15 4·76 3·86 3·68	106 76 89 76 104 109 100 86 83	3·29 3·97 3·49 3·60 2·86 2·37 2·62 3·11 3·08	3·2 3·8 4·3 3·0 3·0 3·6 3·6	·648 ·533 ·493 ·569 ·695 ·618 ·678 ·587 ·566	·517 ·599 ·543 ·556 ·456 ·375 ·418 ·493 ·489
*IIa6 (68)	Rheumatic mitral and aortic dis.	3·26	79	4·00	3·8	·513	·602
†IIa7 (62)	Hypertensive failure	3·94	82	2·91	1·4	·595	·464
IIa8 (67)	Rheum. aortic dis. Fibrillation	3·14 3·01	88 64	4·55 5·39	1·55 2·75	·497 ·479	·658 ·732
*IIb1 (67½)	Syphilitic aortic incomp.	3·7	87	3·18		·568	·502
*IIb2 (67)	Cardiovascular syphilis	2·93	77	2·95	2·7	·467	·470
*IIb3 (65½)	Rheum. mitral dis. Fibrillation.	3·66 4·06	79 76	3·04 2·81	2·0	·563 ·608	·483 ·449
*IIb4 (65)	Hypertensive failure.	3·96	80	3·2		·598	·505

* Case previously described in Quart. J. Med. 1938, 7, 331.

† „ „ „ „ Edin. Med. J. Trans. Med. Chir. Soc. 1938, 171.

‡ „ „ „ „ Lancet 1937, II, 437, Case 4.

TABLE IV (contd.).

Case and height (ins.)	Diagnosis.	V.E. CO ₂	A.V. O ₂	Abs. C.O.	V.C.	Log. V.E.	Log. C.O.
*11b6 (68)	Rheum. mitral dis. Fibrillation.	3.52	84	2.78	2.6	.546	.444
		3.97	119	1.86	2.2	.599	.269
		3.97	89	3.14	2.2	.599	.497
		3.18	104	2.69	2.15	.502	.430
*11b7 (55)	Rheum. mitral dis. Fibrillation.	4.94	90	1.69	1.0	.694	.228
		4.05	71	2.33	1.0	.607	.367
		3.21	72	2.66	1.2	.507	.425
		3.66	75	2.08	1.3	.563	.318
		3.46	51	2.66		.539	.425
		3.55	65	2.28	2.1	.550	.358
*III 1 (56)	Rheumatic mitral stenosis.	4.37	108	2.08		.640	.318
*III 3 (67)	Hypertensive aur. fibrillation.	3.49	90	3.38	1.4	.543	.529
		4.00	78	3.17	1.2	.602	.501
		4.7	120	2.66	1.3	.672	.425
		3.6	78	3.37	2.1	.556	.528
		3.31	65	4.20	2.0	.520	.623
		2.92	67	4.25	2.2	.465	.628
		2.85	63	5.10	2.8	.455	.707
		3.90	86	3.12		.591	.494
		3.29	90	3.80	2.7	.517	.580
		2.49	77	4.60		.396	.663
		2.59	68	3.90		.413	.591
		2.66	59	4.92	2.9	.425	.692
		2.57	71	4.33	3.0	.410	.636
*III 4 (67)	Cor. atheroma (after recovery)	3.75	79	3.38	2.0	.574	.529
*III 5 (63)	Rheum. mitral stenosis.	4.75	138	1.91	1.4	.677	.281
		4.97	153	2.34	1.4	.696	.369
		4.77	114	2.20	1.4	.678	.342
*III 6 (64)	Hypertensive aur. fibrillation.	3.65	156	2.2	1.3	.562	.342
		4.1	96	2.8		.613	.447
		3.54	108	2.50		.549	.398
		3.30	61	4.28		.519	.631
‡III 7 (70)	Rheumatic aortic disease.	5.46	149	1.70	1.75	.737	.230
		6.32	180	1.31	1.9	.801	.117
		6.59	187	1.41		.819	.149
		5.81	157	1.71		.764	.233

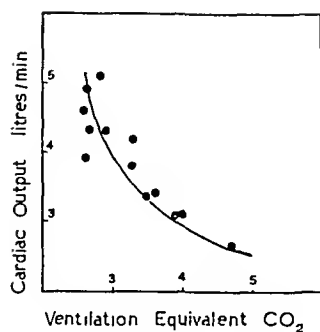


Fig. 3.

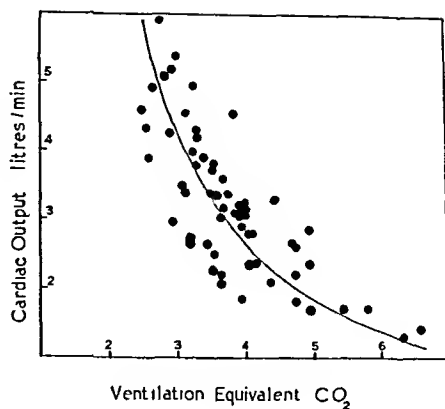


Fig. 4.

Fig. 3. Cardiac output and pulmonary ventilation in Case III 3. There is an apparent *curvilinear correlation*.

Fig. 4. 61 simultaneous observations of cardiac output and pulmonary ventilation are plotted. There is a high degree of correlation. The equation of the line is $x = 7.24/y^{.60}$, corresponding to the straight line in Fig. 5.

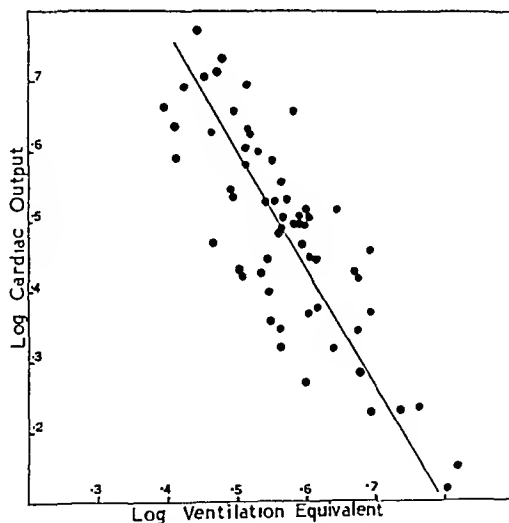


Fig. 5. The logarithms of cardiac output are plotted against log ventilation equivalent ($r = -0.83$). The line is the average of the two regression lines.

Some useful points emerge from a consideration of the quantitative relationship existing between cardiac output and pulmonary ventilation.* This relationship becomes rectilinear when the logarithms are graphed (Fig. 5). Working back from the equation of the average regression line we find that the ventilation equivalent is inversely proportional, roughly, to the square root of the cardiac output.

$$V.E. = K/\sqrt{\text{cardiac output.}}$$

If the rate of CO_2 production of the brain is constant the CO_2 content of the cerebral venous blood will be inversely proportional to the rate of blood flow. Through the physiological ranges with which we are dealing, the CO_2 dissociation curve, and the respiratory response to CO_2 tension may be taken as nearly linear (12, 23). Hence pulmonary ventilation would be expected by theory to be nearly inversely proportional to cerebral blood flow,

$$V.E. = k/\text{cerebral blood flow.}$$

From these two equations,

$$\text{cerebral blood flow} = C.\sqrt{\text{cardiac output.}}$$

This means that when the cardiac output falls from four litres to say, two litres, the cerebral blood flow will fall on the average from $(\sqrt{4})$ 2 units to $(\sqrt{2})$ 1.4 units. In non-quantitative terms, when the general systematic flow is depressed various physiological mechanisms work in favour of the brain, the circulation through which does not fall to the same degree.

This finding, even though approximate, is of great assistance in approaching the quantitative data available on the cerebral blood flow. It is worth keeping in mind that even in the last stage of heart failure (Grade III) the systemic flow is, on the average, about half the normal at rest (17 and 18). It is therefore not to be expected that the cerebral circulation would fall below about 70% of its normal level. Taking into account the normal variations, it is obvious that, from the statistical viewpoint, depression

* An equation giving the approximate relations of the ventilation equivalent and the cardiac output is found in the following manner. The curvilinear relationship expressed by the general equation $y = Kx^n$ (where n is positive or negative) becomes a straight line when $\log x$ is plotted against $\log y$, as it becomes $\log y = \log K \pm n \log x$. The coefficient of correlation between $\log y$ (cardiac output) and $\log x$ (ventilation equivalent) is -0.83 , standard error $.04$, while the correlation ratios $\eta_{xy} = .865$ and $\eta_{yx} = .810$. This close agreement of r and η indicates a straight line relationship.

The regression equations are :—

$$\log y = 1.287 - 1.41 \log x \quad . \quad . \quad . \quad . \quad a$$

$$\log x = 0.810 - 0.492 \log y \quad . \quad . \quad . \quad . \quad b$$

As both x and y are subject to a similar error of measurement the average of these two regression lines may be taken :—

$$\log x = 0.86 - 0.60 \log y$$

$$\text{whence} \quad x = \frac{10^{.86}}{y^{.60}} = \frac{7.24}{y^{.60}}$$

Thus the ventilation equivalent (x) is approximately inversely proportional to the square root of the cardiac output ($y^{.5}$). Regression equation b actually gives a very close approach to this relationship.

of the cerebral circulation will be more difficult to establish than a fall in the systemic minute volume.

Observations on cerebral blood flow.

The most formidable evidence against the idea that diminution in the rate of blood flow through the respiratory centres is the cause of cardiac hyperpnœa comes from the work of Harrison. From his observations on the oxygen content of the blood in the internal jugular vein he concluded that there was no evidence of slowing of the cerebral circulation. The measurements, however, were made in a small series of cases and failed to take fully into account the normal variations. The writer has made observations on the gaseous content of the internal jugular venous blood in four cases. The results are tabulated along with the more significant data of Harrison in

TABLE V.

Percentage oxygen saturation of jugular venous blood. (Data of Calhoun, etc. (3), Cullen, etc. (8), and personal observations.)*

Case No.	Percentage saturation jug. venous blood.	A-V.O ₂ difference jug. vein.	Pulmonary ventilation litre/sq. m. body surface.	V.E. CO ₂
*E.G.	54.4	†7.12	4.58	
*R.J.	62.4	†5.79	4.12	
*E.M.	59.8	†6.42	5.30	
*E.G.	67.5	3.62	5.44	
*C.M.	42.6	7.75	5.56	
*D.W.	39.1	7.45	6.18	
*A.I.C.	56.4	†5.1	7.0	
*W.J.	36.4	10.53	9.82	
I 3	55.1	8.41		2.79
III 5	19.8	10.02		7.70
III 8 (Hypertensive fibrillation)	43.0	9.96		4.22
III 9 Goitre heart	50.6	†8.13		4.69

† Values calculated on the assumption that arterial blood is 94% saturated with oxygen.

Table V. If, in Harrison's method of measurement, pulmonary ventilation over 5.5 litres per square metre of body surface per minute may be taken as a significant increase, only four of his subjects were hyperpnœic.

From the data of Lennox (15) only three out of 167 normal individuals had a jugular venous oxygen saturation below 50%. If we take a ventilation equivalent over 4 to be significantly above normal, and add the data from Harrison's hyperpnœic cases, we may construct a fourfold table as follows :—

Jugular venous blood per cent. O ₂ saturation.	Normals.	Cardiacs with hyperpnœa.
Over 50	161	2
Under 50	3	5

Using Yates' modification of this table for small numbers (19) χ^2 is 59. This leaves no doubt that these marked reductions of the jugular venous blood are abnormal.

Unfortunately 3 cases (A1.C., E.G., and D.W.) in Harrison's series and 1 case (III 5) in the present series had a moderate degree of anæmia. Lennox does not give in his survey the number of "normals" who were anæmic. For this reason a fairer comparison with the normal may be found by studying the absolute arterio-venous oxygen differences. These will obviously indicate changes in the rate of flow. In the data of Lennox the average arterial oxygen content may be taken as 18.25 vols. per cent. (94% saturated). 55% saturation of the jugular venous blood would give an oxygen content of 10.68, and thus an arterio-venous oxygen difference of 7.57 vols. per cent. In Lennox's normal subjects 32 (19%) out of 167 show greater arterio-venous oxygen differences than this. A fourfold table may again be constructed from these data.

Jugular arterio-venous oxygen difference.	Normals.	Cardiacs with hyperpnœa.
Under 7.57 vols. %	135	2
Over 7.57 vols. %	32	5

Again applying Yates' technique, $\chi^2 = 8.05$, which is highly significant.

It is obvious that more data will be required to establish definitely a reduction in cerebral blood flow, as variations in the normal range of values will permit this point to be established only by a statistical study. This preliminary survey of a small number, however, is strongly suggestive that diminished cerebral blood flow does occur in association with cardiac hyperpnœa.

Harrison emphasises the fact that the CO₂ tension in the jugular venous blood is not outside the normal limits. In the three hyperpnœic subjects, III 5, III 8, and III 9, studied by the writer the values of the

CO₂ pressure in the jugular venous blood were 62, 53 and 51 mm. Hg respectively. These results differ from those of Harrison very considerably, and are definitely above the normal range. It is hoped that more figures on this subject may be available in the future.

Discussion.

Since it was advocated by Fraser twelve years ago, diminished cerebral blood flow has been an attractive explanation of cardiac hyperpnœa. As indicated above, the criticisms of this conception have not been based on adequate evidence, and there is in fact a very close correlation between depression of the cardiac output and hyperpnœa. Cardiac output at rest is not very greatly lowered until the later stages of the disease in which hyperpnœa is found to be pronounced.

The absence of any close relationship between vital capacity and pulmonary ventilation indicates that the reflex explanation of the hyperpnœa favoured by recent writers is unlikely to be valid. As pointed out in the introduction the adoption of such an explanation would necessitate the assumption of a reflexly maintained alteration in the sensitivity of the respiratory centre to CO₂. There is as yet no evidence that such a change takes place, and a chemical explanation of the hyperpnœa seems more likely.

It is to be noted that this paper deals strictly with hyperpnœa occurring in cardiac disease *at rest*. Dyspnœa on exertion is the resultant of hyperpnœa and pulmonary congestion. Nevertheless it is likely that at any metabolic rate the cerebral blood flow of the cardiac patient will tend to be subnormal; to this extent it may produce hyperpnœa, and thus play its part in the production of dyspnœa.

SUMMARY.

1. The best available method of standardising measurements of pulmonary ventilation is by the use of a ventilation equivalent. The ventilation equivalent for oxygen is liable to a considerable error, especially with fluctuations in the respiratory quotient. The ventilation equivalent for CO₂ is less liable to fortuitous changes. Normal values of these functions are surveyed statistically.

2. The standard error of the acetylene method of determining cardiac output is assessed as 4·5 per cent. from 85 checked determinations.

3. In heart failure there is no great correlation between hyperpnœa and vital capacity. There is, however, a close correlation between cardiac output and hyperpnœa.

4. When the general systemic blood flow is lowered in cardiac failure there is good reason to believe that the cerebral blood flow is not depressed to the same degree.

5. Consideration of published and personal data on the jugular venous oxygen content supports the hypothesis that the cerebral blood flow is subnormal in hyperpneic cardiac subjects.

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ON THE DISTRIBUTION OF PAIN ARISING FROM
SOMATIC STRUCTURES WITH CHARTS OF SEGMENTAL
PAIN AREAS.*

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Pain from interspinous ligaments.

IN a recent paper (4) I was able to show that pain produced from muscle is always felt diffusely, and is referred upon a specific pattern. Further observations showed that the interspinous ligaments give rise to similar referred pain, and as these structures are situated deep and easily accessible, I decided to map out as completely as possible the various segmental pain areas by stimulating each of these ligaments.

Two prominent vertebral spines were identified by manual palpation, lead and X-raying them, and the interspinous ligaments were stimulated accordingly†; it being assumed that the corresponding ligament was between the spines in the cervical region, but below the spines in the lumbar regions, thus allowing for an 8th cervical ligament corresponding with the 8th cervical nerve. The observations were made under the supervision of other workers in the laboratory.

As in previous observations upon muscle, the pain was produced by injecting 0.1 to 0.3 c.c. of 6% saline into the structure to be tested. The method of injection is simple. Having marked the position to be tested, the overlying skin, fascia and supraspinous ligament are anaesthetised with novocaine as these structures give rise to local pain which may be confusing. The injecting needle is then introduced into the mid-line and passed through the supra-spinous ligament. It is then turned slightly to one side until the tough interspinous ligament is reached at the needle point. At this moment the subject feels a unilateral pain at the point the saline is injected. If the injection is correctly placed the pain is confined to one side of the body, but if the injection is made in the mid-line a bilateral pain is produced.

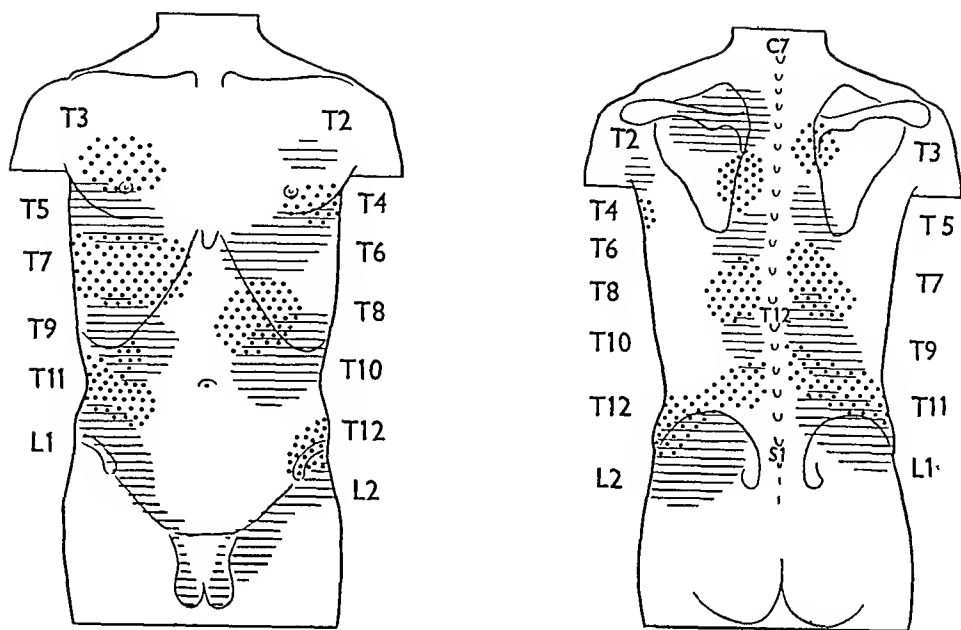


Fig. 1. Shows the distribution of pain arising from the interspinous ligaments T 2 to L 2. Alternate areas have been hatched and stippled.

This ligamentous pain is exactly similar in character to the pain produced from muscle, being a continuous ache lasting from three to five minutes, and described as felt deeply in the limbs and trunk. It is associated with tenderness of the deep structures. The distribution of this deep tenderness corresponds with the distribution of the pain, and its presence enables the subject to mark out the limits of the areas involved with some accuracy. The areas marked out in this way are then recorded on a diagram. Repeated injections of the same ligament gave remarkably constant results, although a period of days or weeks elapsed between the observations.

The interspinous ligaments were injected in turn from the 5th cervical to the 5th lumbar, and a further injection was made over the upper part of the sacrum, this being assumed to represent the 1st and 2nd sacral segments. The lower sacral and coccygeal areas were not obtainable because of the lack of segmental tissue in this region; and the 5th and 6th cervical areas were only obtained with difficulty, because of the depth at which the spines lie in the neck. The areas of pain and tenderness marked out in this way are shown in the accompanying five figures.

The pain areas of the trunk are shown in Fig. 1. This distribution was studied in three different subjects, but there was so little individual variation that one example only has been reproduced.

The pain areas of the arm are shown in Fig. 2. Here the distribution of pain arising from each interspinous ligament is shown separately, and

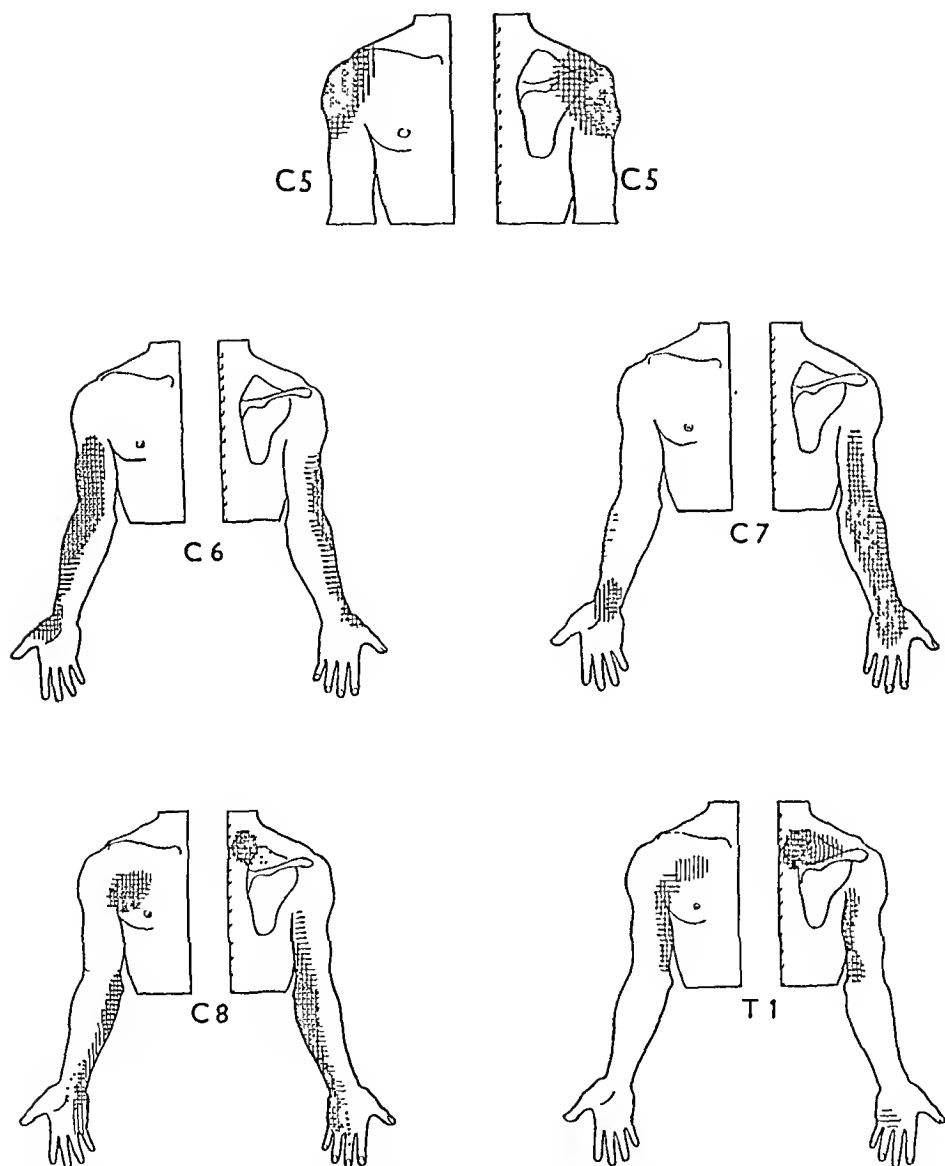


Fig. 2. Shows the distribution of pain arising from the interspinous ligaments C 5 to T 1 in 3 subjects ; vertical hatching, horizontal hatching and stippling.

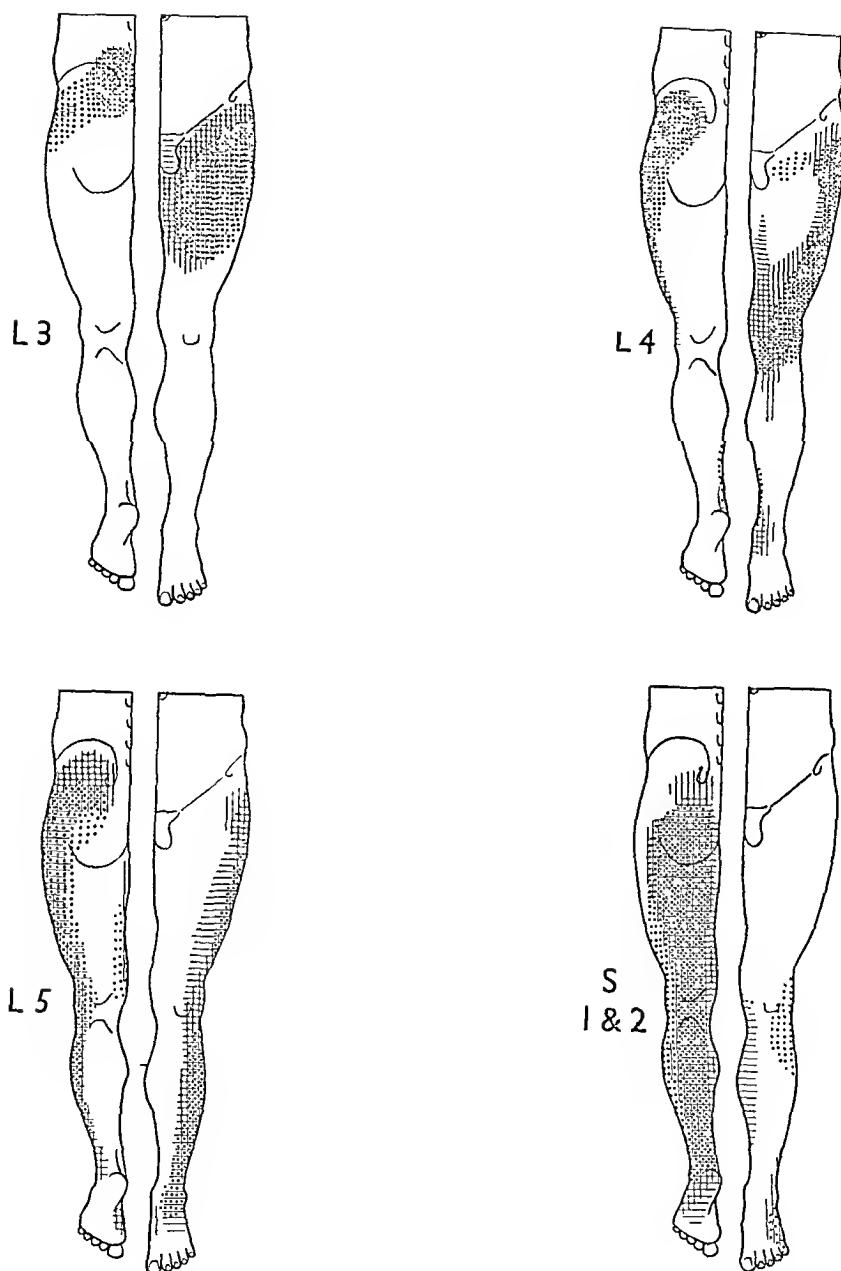


Fig. 3. Shows the distribution of pain arising from the interspinous ligaments L 3 to S 2 in 3 subjects, vertical hatching, horizontal hatching and stippling.

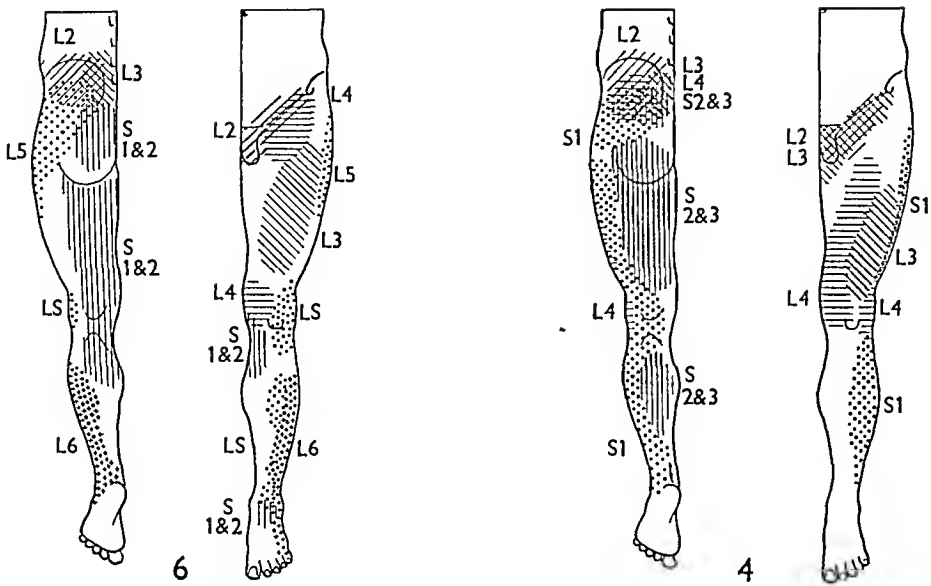


Fig. 4. Shows the distribution of pain arising from the interspinous ligaments L 2 to S 2, in 2 additional subjects, one of whom had 6 lumbar vertebrae while the other had but 4.

represents the superimposed results from 3 subjects. In this way the individual variation is displayed and a common area is obtained where there is overlap. Some of these pains were associated with peculiar sensations in the fingers, but as the subjects had some difficulty in deciding which fingers were involved these sensations were not recorded.

The pain areas in the leg are shown in Fig. 3. Here again the distribution of pain arising from each interspinous ligament is shown separately, and represents the superimposed results from 3 subjects.

The pain areas in the leg were also mapped out on one subject who had 6 lumbar vertebrae, and on another who had but 4 (Fig. 4). These two subjects had their entire spines X-rayed, and they were found to be made up as follows:—The first consisted of 7 cervical, 12 thoracic, 6 lumbar, 5 sacral, and 3 coccygeal; the second consisted of 7 cervical, 12 thoracic, 4 lumbar, 6 sacral and 3 coccygeal vertebrae. It will be noticed that the distribution of the pain areas in these subjects differs considerably from that of the 3 normals. This difference is not simply a caudal or cephalic shift, but is a redistribution of all the areas in the leg. As the frequency of such abnormalities is said to be 6% (1) these differences may be of some clinical importance.

The pain areas in the arm arising from the interspinous ligaments of another subject are shown in Fig. 5 for comparison with the areas of pain which were previously (4) mapped out from his arm muscles. It will be

noticed that although the general pattern obtained by the two methods is similar, there are considerable differences, particularly in the eighth cervical and first thoracic areas. In mapping out pain areas from the arm muscles it was assumed that certain muscles were innervated from a known single segment. This is, of course, only approximately true, and in this subject it is clear that the distribution of pain arising from the interosseous muscle resembles the distribution of T.1 rather than C.8, which it was at first assumed to represent. This subject is also unusual in that he consistently gives smaller areas of pain than the other subjects.

Comment. The distribution of the segmental areas obtained from the interspinous ligaments does not correspond exactly with the distribution of the dermatomes as demonstrated by Foerster (2) or with the areas of skin tenderness recorded by Head (3), and I suggest that the distribution of these areas of deep pain and tenderness may correspond with the distribution of the segmental innervation of some of the deep structures.

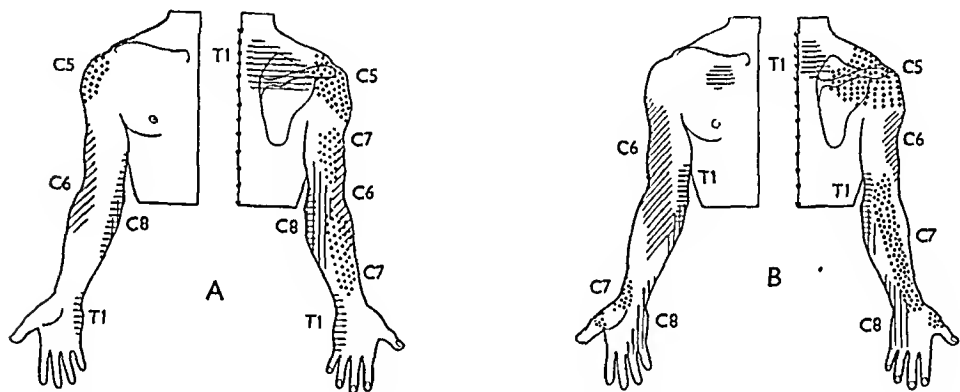


Fig. 5. A. Shows the distribution of pain arising from the interspinous ligaments C 5 to T 1 in another subject.

B. Shows the distribution of pain arising from his arm muscles. Crosses pain from rhomboids; oblique hatchings from flexor carpi radialis; stippling from abductor pollicis longus; vertical hatchings from third dorsal interosseous; horizontal hatching from first intercostal space.

Pain from other deep structures.

The segmental pain areas arising from the interspinous ligaments having been mapped out, the next step was to determine to what extent pain arising from other deep structures is referred over these areas or felt locally. The distribution of muscular pain had been determined previously (4) so that only the pain arising from structures such as the fascia, periosteum, tendons, and joints remained for investigation. The pain was again produced by injecting small quantities of hypertonic saline into the structure to be tested. Membranes such as fascia and periosteum were also stimulated by scratching them with the needle point as by this method alone could one be certain of

stimulating the membrane itself without surrounding structures. Unfortunately the pain produced by scratching with a needle is only of momentary duration, so that although it can be recorded as felt in a certain region, it is too fleeting to allow the limits of its distribution to be mapped out.

The following method was therefore employed. A hypodermic needle was passed through anæsthetic skin and made to impinge upon the portion of fascia or periosteum to be tested. The membrane was then scratched with the needle point, and the region where the subject felt pain was marked

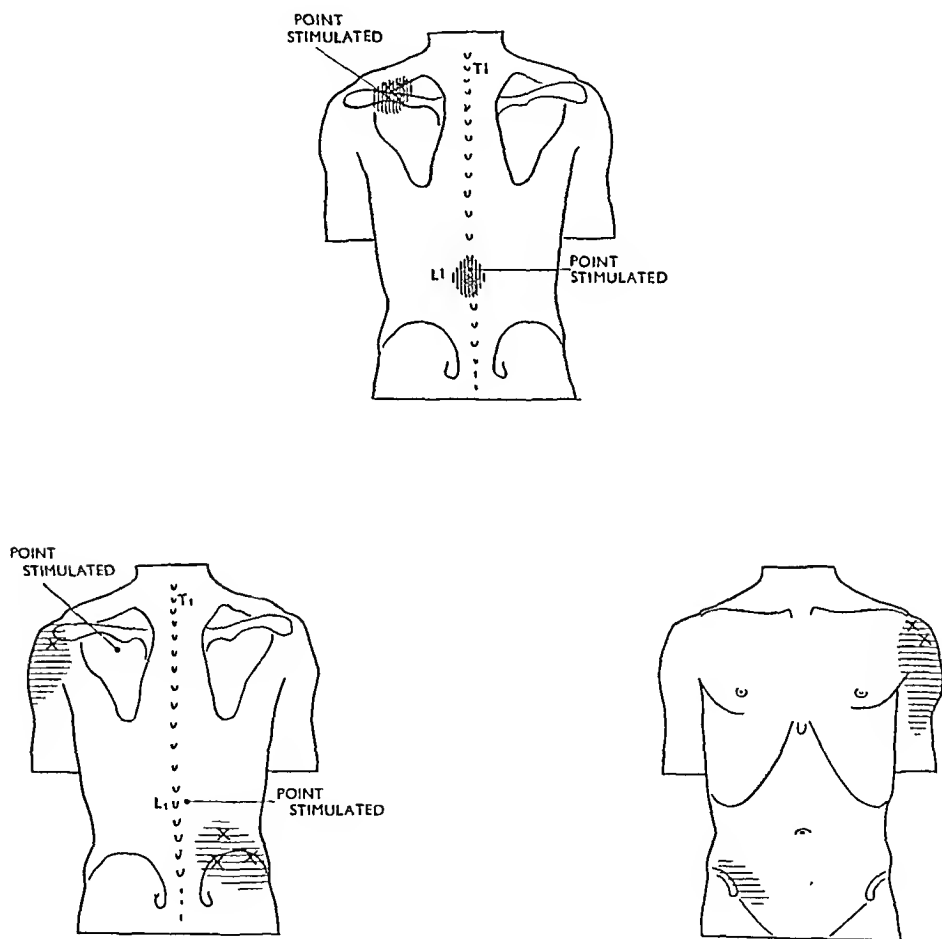


Fig. 6. Shows the distribution of pain produced by saline from subcutaneous periosteum (vertical hatching) and deeply situated periosteum (horizontal hatching). Points stimulated are tip of spine and lamina of first lumbar vertebra and the spine and infraspinal fossa of the scapula. The crosses indicate where pain was placed when the periosteum at the site of injection was scratched with the needle point (3 observations).

with a cross. This was repeated at least three times; after which saline was injected and the distribution of the resulting pain was mapped out. Fig. 6 illustrates the type of result obtained when different portions of periosteum are stimulated in this way. It will be seen that pain arising from subcutaneous periosteum is confined to the neighbourhood of the point stimulated, while pain arising from deeply situated periosteum is felt diffusely and may be referred. It will also be noticed that when saline gives rise to pain which is more or less local, the momentary pain produced by the needle point is regularly placed at the same spot; but that when saline gives rise to diffuse pain, the pain produced by the needle point is placed in various situations, but always somewhere within the distribution of the saline pain.*

Numerous observations of this kind were made on different subjects; but as it would be too tedious to record all of them in detail, the general results will be described and illustrated by a few typical examples.

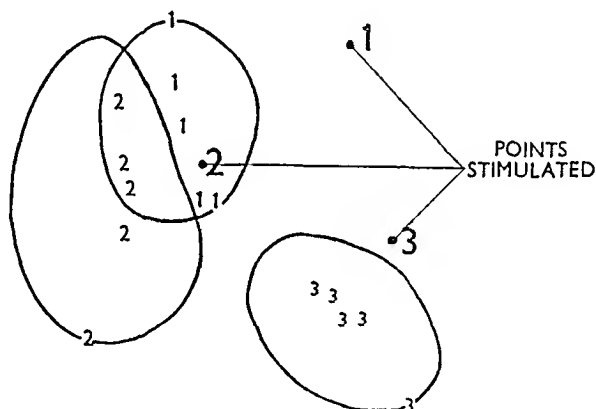


Fig. 7. Is a diagrammatic representation of pain arising from 3 points (1, 2 and 3) on the gluteal fascia 4 cm. apart. Small numbers show where momentary pain from corresponding needle point was placed; circles indicate the limits of pain from saline injected at the same point. (Skin localisation was accurate to within 1 cm.).

Fig. 7 illustrates the distribution of pain arising from deep fascia. It will be seen that the pain produced by saline is not felt diffusely but is confined to a small region, and that the pain produced by the needle point is regularly placed within the distribution of the corresponding saline pain. The pain, however, is not always placed over the point stimulated. On the other hand it is not felt at any great distance from that point, so that although this pain is not accurately localised to the point stimulated it may still be called local as distinct from diffuse in distribution.

Local pain of this type is obtained from all the deep fascia covering the trunk and limbs and also from subcutaneous areas of periosteum such as are

* When a needle is driven firmly into bone it gives rise to an unpleasant sensation of pressure. This sensation appears to be accurately localised, and should not be confused with the pain produced by scratching the periosteum.

found on the tibia, patella, sternum, vertebral spines, acromion, olecranon and phalanges. Subcutaneous ligaments and tendon sheaths such as those at the ankle and wrist, and tendons such as the patellar tendon and tendo achilles also give rise to this type of local pain though the pain may be felt over a larger area when it is severe. On the other hand the intermuscular planes of fascia and deeply situated periosteum and ligaments do not give rise to such local pain but to diffuse pain. From this it is clear that whether pain arising from a given structure is felt diffusely or is confined to the region of that structure depends more upon whether the structure stimulated lies superficially or deeply, than upon its nature (whether fascia, ligament or periosteum). This is well illustrated by pain arising from the chest wall (Fig. 8). Here local pain is only obtained from the fascia and periosteum which is subcutaneous in situation while all the structures situated more deeply give rise to diffuse pain of segmental distribution, whether the structure stimulated be muscle, ligament or periosteum.

In some regions the segmental distribution of diffuse pain may be modified by a crude attempt at localisation. For instance, pain arising

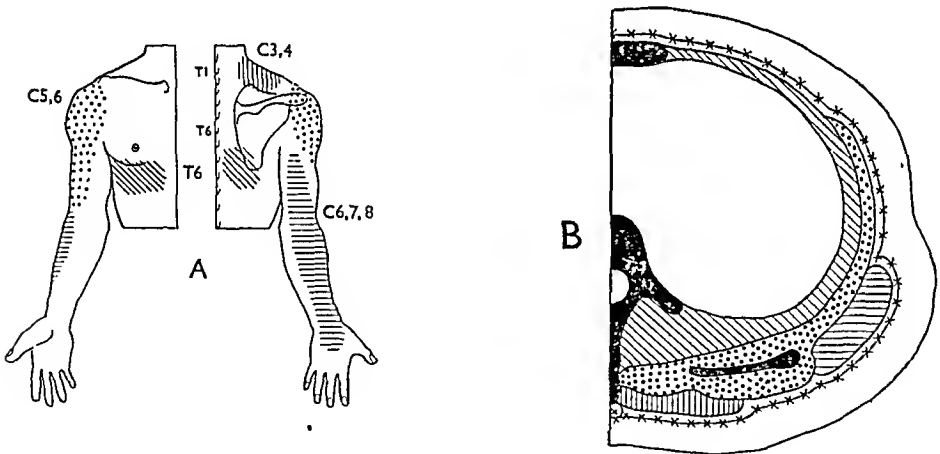


Fig. 8. A. Shows the distribution of pain arising from the various deep structures of the chest wall at the level of the 6th intercostal space. B. shows the tissues which give rise to the corresponding areas of pain. Oblique hatching, represents the intercostal space and erector spinae (T 6); stippling, the muscles attached to the scapula, (C 5, 6); horizontal hatching, latissimus dorsi (C 6, 7, 8); vertical hatching, trapezius (accessory nerve and C 3, 4). Crosses represent tissue giving rise to local pain which is not shown in A.

from the erector spinae may be felt more in the back than the front, while pain arising from the rectus abdominis is felt more in the front than the back. The segmental distribution of pain arising from the limb muscles is modified to a greater extent. Thus pain arising from the anterior crural muscles is felt maximally in front of the ankle, and pain from the extensors of the fingers is felt over the dorsum of the hand; similarly pain arising

from the long flexors of the fingers is felt maximally in the region of the wrist and knuckles. In general there is a tendency for pain arising from the limb muscles to be placed in the region of the joints which are moved by these muscles, provided these joints lie within the segmental pain areas corresponding to the nerve supply of the muscles in question.

In pain arising from the limb joints themselves localisation is more accurate. Fig. 9 illustrates the distribution of pain arising from different parts of the knee. Although the distribution of these pains follows the distribution of the segmental pain areas to some extent, there is in all of

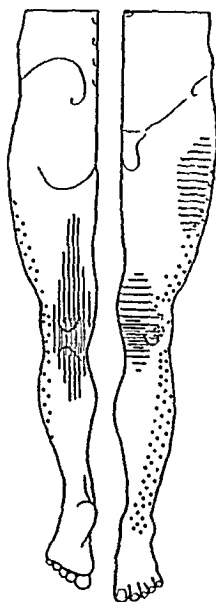


Fig. 9. Shows the distribution of pain arising from structures round the knee. From medial collateral ligament, horizontal hatching; from lateral part of capsule, stippling; from posterior part of capsule, vertical hatching.

them an area of maximal pain in the region of the knee, and when the pain is of slight intensity this area of maximal pain may alone be appreciated. Similar results are obtained from other joints, though pain arising from the joints of the hand and foot is more local, while pain arising from the hip and shoulder is more segmental in distribution.

With the stimuli used I was unable to produce any pain from either articular cartilage or compact bone, though cancellous bone gave rise to diffuse pain similar to that arising from the other deep structures.

Articular cartilage was investigated during the aspiration of fluid from distended knee joints. Whenever the aspirating needle was driven into the cartilage covering the lower end of the femur or the patella the subject experienced a sensation of "tapping" or "pressing" but no pain; but whenever the needle impinged upon the lining of the suprapatellar pouch

the subject experienced severe pain felt "somewhere in the knee." This result was obtained from 4 subjects.

Bone was investigated in the following way. One of my surgical colleagues drove a Kirschner wire through the upper end of my own tibia, after the overlying skin and periosteum had been thoroughly anæsthetised with novocaine. While the wire was passing through the compact bone I experienced a sensation of pressure and vibration but no pain, but when the wire entered the soft cancellous bone diffuse pain was added to the sensation of vibration. The wire was then replaced by a hypodermic needle and 0.1 c.c. of 6% saline was injected into the cancellous bone. This also gave rise to slight diffuse pain felt widely in the outer side of the leg.

Discussion.

From these observations it would seem that beneath the skin there is a second sensitive layer in which pain is localised with fair accuracy. This layer consists of the deep fascia encasing the limbs and trunk and any periosteum, ligament or tendon sheath which is situated subcutaneously. On the other hand all the structures deep to this layer give rise to diffuse pain of more or less segmental distribution. The pain is fully segmental in distribution when arising from the interspinous ligaments, intercostal spaces, and other structures situated deeply in the trunk and limb girdles; while the pain is more local when arising from the extremities, the joints, and the less deeply placed structures in the limbs and trunk.

Pain is usually considered to be of two types "local" and "referred"; the latter having a segmental distribution and a special neurological mechanism. Pain arising from the somatic deep structures, however, presents a gradual transition from pain which is confined to the region of the structure stimulated to diffuse pain of full segmental distribution, and in either case the situation of the point stimulated may or may not lie within the distribution of the pain. Thus a classification into "local" and "referred" pain cannot be applied consistently. Instead we have to speak of pain which is moderately well localised, and diffuse pain which is poorly localised.

The better localised pain is obtained from the more superficial body coverings, and from the limb joints and other structures of which we are conscious as a result of palpation and movement, while diffuse pain is obtained from the more deeply situated structures of which we are ordinarily unconscious. This diffuse pain appears to be projected to the region of those deep structures in which pain is well localised and which are innervated by the same spinal segment as the structure stimulated; in this way the pain is given its segmental distribution. Thus, the segmental distribution of diffuse pain may simply be a form of false localisation.

SUMMARY.

1. Segmental areas of deep pain and tenderness have been mapped out by stimulating the interspinous ligaments.

2. An extensive investigation has been made of the distribution of pain arising from the various deep somatic structures, and it has been found that they give rise to pain, the distribution of which presents a gradual transition from pain which is confined to a spot in the region of the structure stimulated, to diffuse pain of full segmental distribution.

3. Whether the pain is local or segmental in distribution appears to depend more upon the depth at which the tissue stimulated lies than upon its nature (whether muscle, ligament or periosteum).

4. These findings are briefly discussed, and it is suggested that the segmental distribution of diffuse pain may be a form of false localisation.

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OBSERVATIONS RELATING TO REFERRED PAIN, VISCERO-MOTOR REFLEXES AND OTHER ASSOCIATED PHENOMENA.*

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IN a recent paper Kellgren (7) has described the distribution of pain produced by stimulating the interspinous ligaments in man. He has shown that pain arising from these ligaments is distributed upon a well defined segmental pattern. It was recognised that this example of referred pain in the somatic system, associated as it is with deep tenderness, would be given a wider and greater significance if it could be brought into relation with certain other phenomena, and especially if the underlying mechanism could be correlated with that underlying the referred manifestations of visceral disease.

In the original studies in which the interspinous ligaments were stimulated, attention was concentrated chiefly upon the distribution of pain; muscular rigidities and cutaneous hyperalgesia were not noticed. As both these phenomena occur in association with the pain of visceral disease, we determined first of all to search for them as responses to stimulation of the interspinous ligaments. For this purpose 6 normal subjects were used.

RESPONSES TO STIMULATION OF INTERSPINOUS LIGAMENTS IN MAN.

The details of injection have been similar to those previously described. The skin and superficial ligaments are anaesthetised at the chosen spot, and a needle then thrust in until, at a depth of about 1 to 3 cm., the deep ligament is encountered. Carrying the needle a half centimetre to one side of the mid-line usually gives a little preliminary pain that is felt unilaterally. The injection of 0.3 c.c. of 6% saline (or less) is made at once, and the results noted. It is to be remarked that the stimulus is quite local and applied to a small ligament or to muscle fibres attached to it, and the response is invariable and immediate; thus there is no possibility of direct stimulation of spinal nerves. Before the injection is made the subject is examined for hyperalgesia and for any departure of the relevant muscles from normal and symmetrical flaccidity. The responses about to be described appeared in the 6 subjects with considerable uniformity.

1st lumbar. We first chose the 1st lumbar ligament for investigation because it was known (7) to give pain distributed in a manner strikingly

* Work undertaken with the aid of the Medical Research Council.

† Beit Memorial Research Fellow.

resembling that characterising renal colic, namely, pain in the loin, in the inguinal, and in the scrotal regions. Having made the injection with the subject standing we looked first of all, as the pain developed, for retraction of the testicle on the corresponding side. In most subjects this retraction was seen unmistakably and in two subjects it occurred conspicuously. The retraction becomes maximal as the pain swells to its height, and gradually disappears as the pain subsides during the next 3 to 5 minutes. The same injection frequently gives distinctly palpable rigidity and deep tenderness of the lowest part of the abdominal wall of the corresponding side; the local rigidity and deep tenderness pass away as the pain subsides. Testicular tenderness is frequent. Cutaneous tenderness to light friction was found in 4 out of 5 subjects in which it was looked for; its position is exemplified in Fig. 1. It is to be noted that the regions of superficial and deep tenderness do not correspond.

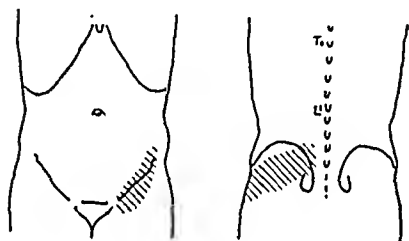


Fig. 1.

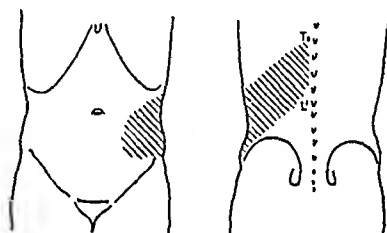


Fig. 2.

Fig. 1. Area of cutaneous tenderness following injection of 1st lumbar interspinous ligament.

Fig. 2. Area of cutaneous tenderness following injection of 9th thoracic interspinous ligament.

9th thoracic. This was our second choice. The results of injection into this ligament have also been uniform. The pain appears over the segmental region already described (7); this is represented by an area in the back opposite the 1st lumbar spine, and another in front passing over the 9th costal cartilage towards the umbilicus. Muscular rigidity, associated with deep tenderness, appears as the pain comes to its height; it mainly involves the upper quadrant of the abdominal musculature on the homolateral side, though slight rigidity may be found on the other side also. When pain is severe the upper belly of the rectus may stand out as an obvious phantom tumour. The subject is often conscious of the unilateral spasm of the abdominal muscles, and of a desire to cease breathing owing to a sense of fixation of the chest. Flattening of the lower ribs and diminished movement, on the affected side, may be clearly visible. These signs disappear hand in hand with the cessation of pain. Cutaneous tenderness in some degree was detected by all 6 subjects. Its distribution is represented by Fig. 2. Skin tenderness is not detected until about 5 min. after the injection, when pain has usually gone; it is very slight at first, but develops

to become clearer, and it often lasts for considerable periods, such as one to several hours. The degree of cutaneous tenderness observed has varied in different instances but usually it has been slight; it is elicited by light friction or by pinching up the skin. Its distribution is not the same as that of the deep tenderness; the latter corresponds with the muscular rigidity.

The next two ligaments were chosen to test the spread of objective phenomenon to the tissues of the limbs.

8th cervical. Injection of this ligament gives pain in the upper interscapular region, over the pectoralis major and down the inside of the elbow and forearm. Muscular rigidity has not been detected, though there is a sense of constriction in the upper chest of the affected side. Skin tenderness is occasionally noted over a small area on the ulnar aspect of the wrist.

1st and 2nd sacral. The injection is made into the periosteum over the upper part of the sacrum. Pain occurs in the buttock and down the back of the thigh and calf. No palpable rigidity of muscles has been detected, but a characteristic posture is assumed, while the pain is at its height, all the joints of the leg being a little flexed, the toe resting on the ground, and the subject being unwilling to throw weight upon it. The spine is tilted a little away from the affected side in the lumbar region giving a slight scoliosis. Skin tenderness sometimes appears over the upper and medial portion of the buttock.

Comment.

It will be clear from these examples that by injecting in the appropriate place such somatic structures as the interspinous ligaments with a little hypertonic saline, it is possible to reproduce pain having the segmental distribution characteristic of visceral disease, simultaneously with the superficial and deep tenderness, and the muscular rigidity, which frequently accompanies such pain (the viscerosensory and visceromotor reflexes of Mackenzie (12 and 13)). The unilateral rigidity of abdominal muscles, and the retraction of the testicle are the more remarkable because they cannot be brought about by voluntary effort. As in visceral disease the rigidity and skin tenderness are more in evidence upon the trunk than upon the limb. A manifest and simple explanation of these facts is that there is a common, though complex, mechanism, stirred into activity by afferent impulses derived either from deep lying somatic structures or from disturbance of a viscus.

COMPARISON OF CHARACTER OF PAIN OF DEEP SOMATIC AND OF VISCERAL ORIGIN.

We know from our personal experience that the pain produced by injections of saline into the ligaments of the spine has certain characteristics. At its height the pain is unvarying, continuing smoothly at one degree of intensity; and it has a peculiar but indescribable quality, quite distinct from burning pain derived from skin or mucous membrane, but similar to

that of pain derived from other deep lying somatic structures. In the observations to be described we have tried to compare this pain from deep somatic structures with pain of visceral origin. For this purpose we have chosen patients who suffer frequently from visceral pain, and who are sufficiently interested to give close attention to what is happening. It is very important that such subjects should be entirely willing to co-operate and at their ease, and that they should be made to appreciate before hand precisely the points at issue. Most of our subjects have been cases of angina of effort in which the pain is unilateral and felt, at first or at last, down the inner side of the left arm or forearm. Such a subject is asked to recall his pain as clearly as he can, and is the more able to do so if he has suffered (or been induced to suffer) from it quite recently ; he is then asked to compare this pain, to which he is used, with the pain provoked by injecting the left interspinous ligament lying below the 7th cervical or 1st thoracic spines. Such injection gives pain in the back and down the inner side of the arm or forearm, and he is asked to tell us quite frankly if the two pains are different, similar, or indistinguishable in quality. We have made these tests on 4 subjects, including a medical colleague who has long been very interested in the mechanism of anginal pain. There seems to be no purpose in describing these cases in detail. They were all middle-aged or elderly men in whom the diagnosis of angina of effort could scarcely be questioned, owing to the character and distribution of pain in them, its invariable provocation by effort, and its quick relief by nitrites. From all these subjects we have obtained similar and to us convincing answers, the pain from injection of the spine is described as being of exactly the same kind as that felt in attacks produced by effort. The distribution is not identical, since the injection provokes pain in the back near the site of injection, while in the anginal attacks pain in the front of the chest is often more prominent. But apart from these natural differences it has been clear that the subjects themselves have been definitely impressed by our ability to induce a pain, which in its onset, continuation and character, closely resembles the pain of which they come complaining. In more than one case too the pain from the injection has been accompanied by a feeling of numbness or tingling in the left hand, or by subsequent hyperalgesia of the skin of the upper arm ; and in detail these associated symptoms have repeated those of the anginal pain of effort in the same subjects.

In the medical man in whom angina has been experienced as bilateral pain over the sternum, injection of the 3rd thoracic spinal ligament in the middle line has brought corresponding pain, stated to be very similar to that previously experienced and associated with a similar sense of constriction in the upper chest.

Both of us suffer not infrequently from intestinal colic, and this pain has been compared with that produced by injecting the salt solution into the belly of the rectus muscle just below and an inch outside the navel. This injection gives a continuous pain lasting 3 to 5 min. of unpleasant severity and

having a character not to be distinguished from that of colic; though naturally its time-intensity curve is different. The pain too is diffuse in character and is located in the front of the body, but deeply below the surface of the abdomen, as it is in colic.

Comment.

In the light of these comparisons we are disinclined to entertain the view that pain derived from deep lying somatic structures is distinct in character from that to which visceral disturbance gives rise.* Although in practice the pain of visceral disease can usually be distinguished from that of somatic disease by its time-intensity curve, by its distribution, and by the associated phenomena displayed in the body wall, it would seem from our present observations to be suggested that none of these criteria is absolute, but rather that every feature and association of the main symptom pain may be experienced whether it is derived from an abdominal organ or otherwise.

THE MUSCULAR REFLEX OF SOMATIC AND VISCERAL ORIGIN. ~

The comparison of pain arising out of somatic stimulation and visceral disease is admittedly a subjective comparison and difficult to guard from error. We have attempted to render it legitimate by insisting on the comparison being made on subjects experiencing both types of pain within a short space of time and in a given part of the body. A second comparison is possible and this can be made strictly objective, namely, that of the muscular response when this is derived from the somatic or visceral structures. For this and for other purposes we have used animal experimentation.

Under full ether anaesthesia the cat is decapitated by Sherrington's method (21). The cat is allowed to lie on an unwarmed table for an hour or a little less so that the ether may be eliminated and the cord allowed to recover from its transection. It is important to bring the cat's reflexes to a suitable point of activity. Warming the cat, so that its rectal temperature remains above 37°C, often reduces activity to an undesirable extent; over-ventilation tends to increase activity undesirably. Soon after decapitation scratch reflexes and other movements of the hind limbs are sometimes very much in evidence; these movements, which in the early stage interfere with the reflexes it is desired to record, usually decline or disappear after the cat has been allowed to rest quietly.

The structure stimulated to induce the muscular reflex has varied as will appear. Our records have been taken chiefly from the rectus muscle, the upper abdominal or very occasionally the thoracic belly being used; occasionally the vastus externus below the ribs has been employed. To record the movement Cushny's myograph has been used; the two points

* Pain derived from skin is here definitely excluded, for its character is distinct. The distinction between the character of pain derived from different deep lying somatic structures, such as tendon and muscle, originally discussed by one of us (11), is one which we find it increasingly difficult to recognize.

of the apparatus are sewn directly to the muscle, exposed through small apertures in the skin and subcutaneous tissues. This instrument has the advantage of responding very little to any movement of surrounding structures; thus, while a contraction of the rectus produces a large excursion, movements of the legs such as are seen in the scratch reflex have little effect on the record.

Responses from the back.

Salt injection. When 6% saline is injected into the interspinous ligament or into the muscles of the human back in the vicinity of the ligament, the muscles of the abdomen that respond do so by entering a state of tonic contraction. In this the contraction resembles that seen clinically in visceral disease.

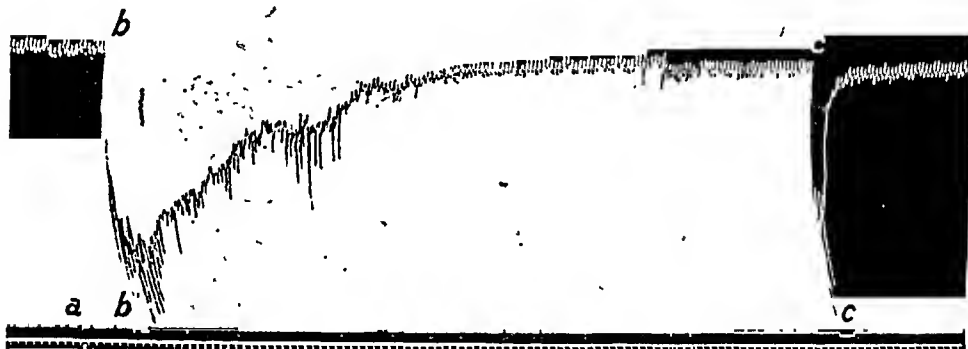


Fig. 3 $\times \frac{1}{2}$. Cat. Record of contraction of right abdominal oblique muscles (mid-region) *a*, insertion of needle into muscles of back, right side, at level of 13th thoracic spine; *b*, injection of 0.1 c.c. 10% saline. *c*, after the index marks, shows the response of the abdominal muscles to a pinch of the same dorsal muscles. Time in this and subsequent figures in 5 sec. intervals.

In the decapitated cat 0.1 c.c. of 10% saline injected into the back a little to the side of the middle line and about the level of the 12th or 13th thoracic vertebra* produces an almost immediate contraction of the upper belly of the rectus abdominis and of the vasti in the upper and lateral parts of the abdomen. The contraction is long sustained, lasting for as much as 5 or 10 minutes, though it declines in degree as it proceeds, and the curve in its last stages returns very slowly to the original level. In addition to the main tonic contraction, the muscle twitches, and these twitches superimpose many or occasional spikes upon the curve (Fig. 3). Thus the response in man and in the decapitated cat are very similar, differing only in the presence

* This level is suitable to obtain the reaction described, but the injection may be made at different levels, and will give response in corresponding groups of muscles.

of the little twitches, which may be due perhaps to the freedom of the cord from control of upper centres, in the latter. In stimulating the ligaments and muscles in man it is very convenient to use the saline injection; though this cannot be repeated satisfactorily more than once or twice at the same spot; it is not the form of stimulus one would first choose in animal experiment.

Pinching. This form of stimulation is valuable because it can be repeated and because its intensity and duration can be varied at will, and because it can be applied to different parts with little or no risk of stimulating surrounding tissue; it is preferable to electrical currents for this reason.

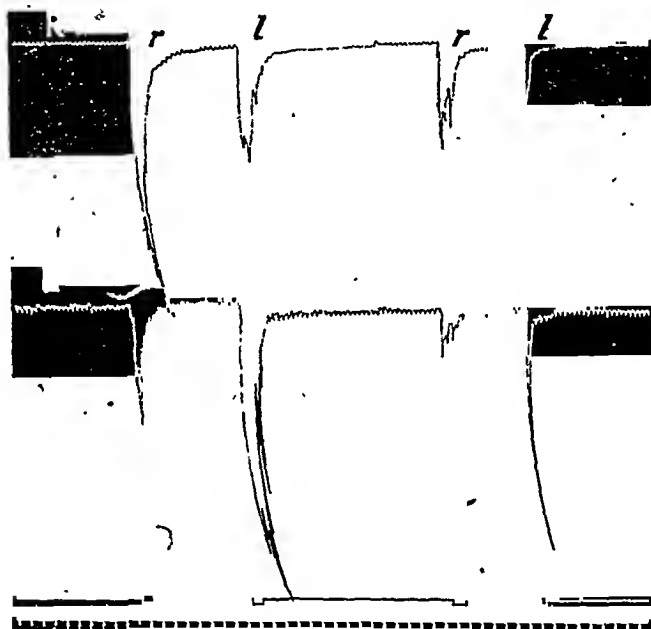


Fig. 4 $\times \frac{1}{2}$. Cat. Records of contractions of the upper abdominal recti (R = right, L = left). The contractions are responses to stimulation (pinching) of the back muscles at the level of 12th thoracic spine. *r*, pinch of muscles on right and *l* of those on left side. The response is bilateral, but much greater on the homolateral side.

If the muscles of the cat's back are exposed and two little slits are made parallel to the midline, the blades of a dissecting forceps may be inserted and the muscle lying between gripped when required. This is a very effective stimulus and produces tonic responses (with superimposed twitches) of the rectus, lasting as long as does the stimulus, if this is of a few seconds duration. Such responses are clearly of the same kind as those produced by saline injections, the latter however being more prolonged, because the stimulus is more prolonged (Fig. 3).

We have used the saline injection to bring the observations on man and cat into line with one another. It is clear that what we have observed in man can be reproduced with sufficient exactitude in decapitated cats; in these it may be further studied and compared with responses from other sources. For these later comparisons we have used the pinch stimulus. The response of the rectus to pinching of the back, as to injection, is sometimes strictly homolateral; more often a lesser response is seen on the contralateral side (see Fig. 4).

Responses from the abdomen.

After Mackenzie had described what he termed the visceromotor reflex, namely, muscular rigidity as an accompaniment of visceral pain, he enlisted Sherrington's help and the latter was able to produce movements of the wall of the abdomen by stimulating the gall duct or by stimulating the central end of the superior mesenteric nerve (personal communication, see also Mackenzie (13)). Subsequently, further work was done by Miller and Simpson (15). These workers have described the development of rigidity of the abdominal wall, in response to traction on stomach or its mesentery, stretching a loop of small intestine, stimulating the central ends of nerves running to stomach and liver, and in other ways. The response to traction on the stomach and its mesentery is abolished or reduced by section of the splanchnic nerve and is greatly reduced by section of appropriate dorsal roots; thus it is obvious that the afferent impulses pass by way of the splanchnic nerves and of posterior roots to the cord. When we studied the published curves, the resemblance to our own curves provoked by stimulating the dorsal muscle was already obvious, but we determined to make similar observations for two reasons. Firstly, that by taking curves derived from back and viscus side by side in one and the same animal, we might more accurately compare them. Secondly, because we were not content that responses to the forms of stimulation used by Miller and Simpson, for instance traction on organs and massage of them, are always open to their interpretation; especially we desired to ascertain by clearer evidence if the visceromotor reflex could be obtained directly from a viscus, rather than from structures attached to it. Our results have differed in certain respects from those of these workers. The relevant facts will be described in some detail.

All the observations now to be described were done on animals giving response at the time from the back muscles or from other tissue that ordinarily responds. A conclusion that any tissue fails to give response has never been drawn unless active responses have been obtained from other tissues directly before and after.

To stimulate the viscera it is necessary to expose them, and in doing so the abdominal wall must be broken. It is consequently important to know what effects may arise from the body wall itself.

Skin. Consider first of all the skin with its underlying panniculus carnosus. Rubbing the skin, especially over neck and shoulders, gives the well known scratch reflex. Cutting or crushing the skin is accompanied by kicking movements, usually vigorous, in which the hind leg is first flexed and then sharply extended. They may be unilateral or bilateral and have very definitely the appearance of movements intended to fend off the source of irritation, by moving the eat away or by striking an aggressor. The kick is elicited with increasing strength and ease as the skin cuts are placed farther and farther below the level of the 13th thoracic segment. Faradic stimulation of the undermined skin, causes in addition to kicking, widespread contraction of the panniculus. But no form of skin stimulation gives localised contractions of the abdominal wall, though some abdominal wall contraction may happen as part of a vigorous kicking movement. These vigorous reactions from the skin make it important to limit the damage to skin in opening the abdominal wall, otherwise they are apt to persist and interfere.

Muscles, etc.. Stimulation of the branches of the thoracic nerves as they proceed obliquely across the abdominal wall, or of their central cut ends, gives strong reactions in the recti of the same side. So does direct stimulation of the oblique muscles by pinch or faradism. This is precisely in line with the response from the muscles of the back already described. If the peritoneal cavity has been opened, even light friction of the cut edges of the wound may set up vigorous abdominal responses. Great care, therefore, must be taken to avoid such friction when responses from the abdominal contents are being tested.

Movements of the legs occur quite often, but these are usually dissimilar to those already described as derived from skin stimulation. Kicking movements may occur but are not often seen; oftener, there are alternating movements of the two legs as in trotting; the predominant form of response is a slow strong extension of the hind limb, chiefly but not exclusively homolateral. Exactly similar leg responses are seen when the back muscles are pinched, though the form varies. In a given eat, whatever the type of response, it is always the same, whether the back or the abdominal wall is stimulated.

Parietal peritoneum. It is impossible to stimulate the parietal peritoneum mechanically without involving in varying degree the connective tissue and muscles lying outside it. Its sensitiveness has for this reason long been a matter of debate. To test this peritoneal surface the abdominal walls should be opened obliquely in the line of its nerves, so as to disturb them as little as possible; the wall is supported and the exposed viscera gently depressed by suitable retractors, so that an adequate area of peritoneum can be stroked without touching any other structure. The smooth end of a blunt probe, or a small compact mass of cotton wool has been used to stimulate; the latter moves over the surface with more friction than the former and is the more powerful stimulant. The strokes are carried to and fro during a period of stimulation lasting 5 or more seconds; the strokes

are sufficiently firm visibly to raise the whole abdominal wall. Such stroking of the anterior and lateral parts of the abdominal cavity may produce small responses; usually large areas, or even the whole extent of lateral and anterior walls, give no response. The responses are much more regular and they are often much more vigorous when the probe passes beneath the ribs and rubs the under surface of the diaphragm. The central parts of the diaphragm when stimulated provoke little or no response either of abdominal or of thoracic musculature; a probe passed under the ribs, may be rubbed up and down the outer and, alternately, the central parts of the diaphragm giving large reactions from the former and little or no reaction from the latter (Fig. 5). The outer parts of the under surface of the diaphragm often

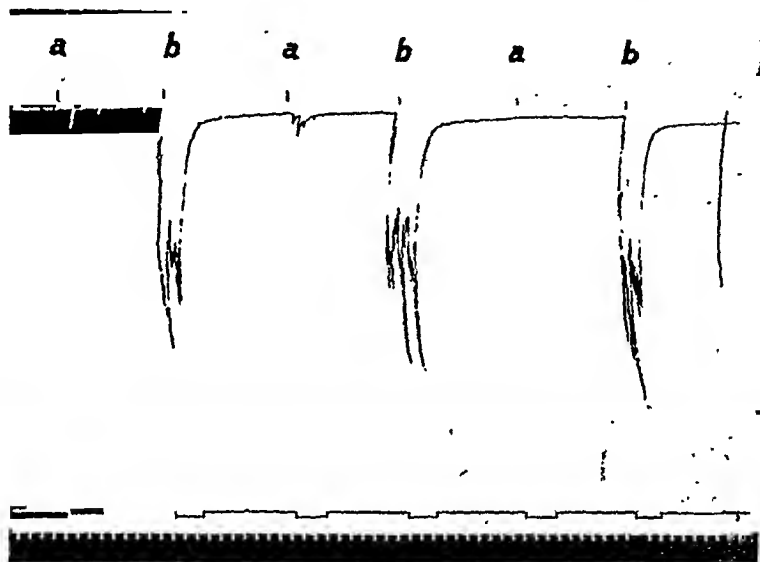


Fig. 5 $\times \frac{1}{2}$. Cat. Record of contractions of right rectus muscle, upper abdomen. Six stimulations of the under surface of the diaphragm on the right side, by drawing to and fro over its surface the smooth round end of a probe. This friction was continued during the whole period of stimulation indicated by each signal. Stimulation at *a* was of the dome and at *b* of the lateral parts of the diaphragm.

yield a single sharp twitch, each time the probe is drawn across them. The ready response to stroking the outer parts of the diaphragm is due chiefly to two causes. These parts of the diaphragm lie against the ribs, which exert counter pressure; thus, if the lateral walls of the abdomen are stroked across a metal rod placed outside, response is then more likely to happen. But the chief reason is that when the probe jumps over a rib it directly stimulates the intercostal nerve; these nerves are very sensitive to mechanical stimulation, as can be shown by directly testing them with the chest open, and strokes of the diaphragm from below against a supporting bar placed above gives a much less certain and less vigorous response than if the support is given by rib. The rib spaces are not particularly sensitive, rubbing the rid

itself longwise shows this to be more sensitive ; pass across it and its nerve, and the reaction is intense, involving several rib spaces and abdominal muscles.

Below the level of the kidneys the posterior abdominal wall gives little or no response.

Briefly, we have not found the parietal peritoneum to have such sensitivity as to be a frequent source of error in testing the viscera, especially as it is the wall unsupported by ribs which is most likely to be touched inadvertently. The cut edges of the wound, against which a viscus withdrawn from the abdomen may rub, are much more likely to give rise to wrong interpretations, and this is especially so if a thoracic nerve lies cut or uncut at the edge of the wound.

Duodenal loop. It was our desire to find some viscus within the abdominal cavity, which could be reached easily and which would give ready and invariable responses. The organs were examined one by one, using the pinch stimulus for the most part. We found that we could never elicit responses from the solid organs, such as kidney, spleen, liver ; neither could we obtain responses from any part of the stomach or bowel, though the pinches might crush the muscle and give hæmorrhage into the wall and were always firm enough to produce strong contraction of the wall. Attempts to obtain responses from the ureter by mechanical stimulation from without, or by inflating it and the renal pelvis have had no regular success.* The perirenal tissues also seem to be insensitive. The Fallopian tubes are very sensitive. The mesenteries of ileum, jejunum, and large intestine yield occasional reflexes, especially when the pinch includes the larger vessels of the mesentery ; reflexes are usual from the mesentery of the cæcum. Pulling on any of these mesenteries usually gives a response. The great omentum has never given response except at its root where it holds the tail of the pancreas. Though crushes of the stomach wall are without effect, handling the organ easily brings responses, presumably by pulling on the mesentery. The gall bladder gives no response, but pinches of the region of the common bile duct give vigorous responses. The stomach and structures lying in the small omentum cannot be well displayed through a small incision and therefore did not prove very suited to our purpose. All these responses consist of similar movements of the abdominal wall, including rectus and oblique muscles. According to the site of the mesentery stimulated the abdominal wall nearer to the thorax or nearer to the pelvis contracts. All these responses may be associated with a similar form of leg movement, usually a slow movement of extension, as when a cat stretches ; but this movement is most readily obtained from organs lying near the pelvis.

After searching we found precisely what we wanted for further work in the duodenum. In the cat this organ forms a single long loop suspended on a long mesentery. The central part of the mesentery holds the main vessels to this gut ; the rest of it contains the long ribbon of pancreatic

* Inflation above 100 mm. Hg is apt to produce embolism of the renal veins.

gland, which runs parallel to the gut over its whole length and also sends a long tail into the root of the great omentum.

Reflexes of the abdominal wall are very readily elicited from this loop. It can be exposed nicely through an incision dividing the abdominal wall immediately ventral to the right kidney and can be withdrawn and suspended outside the cavity by a thread passed through the gut wall, without placing the mesentery under undue tension. The same incision serves to expose and divide the right splanchnic nerve when this is desired.

A large number of very careful observations has been made on this loop, testing chiefly its apical portions, and we remain in no doubt that it is the mesentery with its pancreatic content from which the reflexes are derived. If the gut is pinched firmly it contracts locally, if its wall is incised it contracts similarly, but there is never any response of the abdominal muscles. Electrodes conveying a faradic current easily appreciated by the tongue may be placed on the gut wall and gradually passed along the surface which is opposite to the mesenteric attachment; thus, the bowel wall may be brought to contract strongly over 5 or 6 cm. or more, but no muscle reflexes are set up. If a balloon 4 to 6 cm. long is tied into the gut through an incision in its wall, it may be inflated gradually or abruptly to pressures of 150 or 160 mm. Hg. Such pressures are more than adequate to produce extreme stretching and blanching of the wall, but no abdominal reflex follows, provided care is taken that no drag is placed on the mesentery and that the inflation does not disturb the cut edges of the abdominal wound (Fig. 6). But if, instead of ballooning or pinching the gut, the forceps are moved just a little way onto the pancreas, which lying in the mesentery comes into actual contact with the gut near where this has previously been stimulated, a pinch of this pancreas almost invariably yields a vigorous contraction of the rectus abdominis and the oblique muscles lying lateral to the rectus in the upper part of the abdomen. All parts of the mesentery with its contained pancreas are sensitive in this fashion as is also the omentum where it contains the long tail of the pancreas. The afferent nerves concerned are associated with the pancreas, for in the case of the omentum pinches may be carried up to the pancreas on all sides without provoking a response, which is generally immediate if a fragment of pancreatic tissue is included. Because of this association and for brevity we shall in future refer to stimulation of or response from pancreas.* It is easy to obtain records of vigorous muscular responses from the pancreas, alternating with stretches of curve undisturbed by pinches of the bowel (Fig. 7). A quick pinch of the sensitive tissue yields a single contraction lasting a few seconds, a longer pinch gives a tonic contraction lasting 10 or 15 seconds, on which twitches are superimposed. Very sustained contractions cannot be obtained by pinching, unless the forceps are moved from point to point. The abdominal muscles contract chiefly but not exclusively on the right side.

* Sensitivity to faradism is greatest along the course of the vessels running to the pancreas; the last centimetre of the tail may give no response, but the rest of the organ usually gives ready responses.

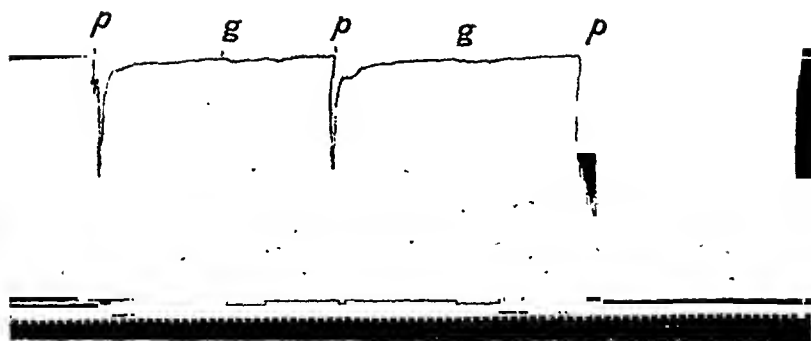


Fig. 6 $\times \frac{1}{4}$. Cat. Record of contraction of right upper abdominal rectus. *p* = pinch of pancreas. *g* = balloon blown to 160 mm. Hg in lumen of duodenum.

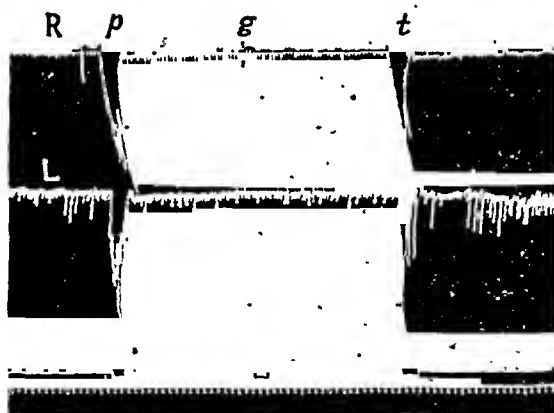


Fig. 7 $\times \frac{1}{2}$. Cat. Record of contractions of the thoracic recti (*R* = right, *L* = left). *p*, pinch of pancreas; *g*, pinch of gut yielding strong contraction of bowel; *t*, tension on mesentery.

Vigorous responses are to be obtained by putting tension on the attachments of the duodenum, but not by stretching a length of the gut between the fingers.

Section of the right splanchnic nerve, which itself yields a very vigorous response, usually abolishes the response from pinches of pancreas in the

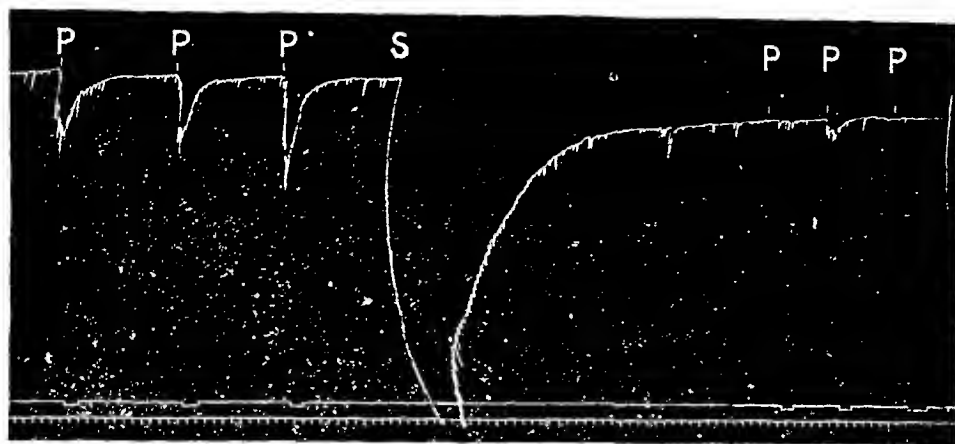


Fig. 8 $\times \frac{1}{2}$. Cat. Record of contraction of right rectus muscle, upper abdomen. The 1st three contractions *p* are responses to three pinches applied to separate bits of pancreas. At *s* the right splanchnic nerve was divided. The pancreas was again stimulated by pinching it three times; at the 1st and 3rd there was no response; the slight response at the 2nd was probably due to a little tension being thrown on the mesentery of the duodenum when the pancreas was picked up.

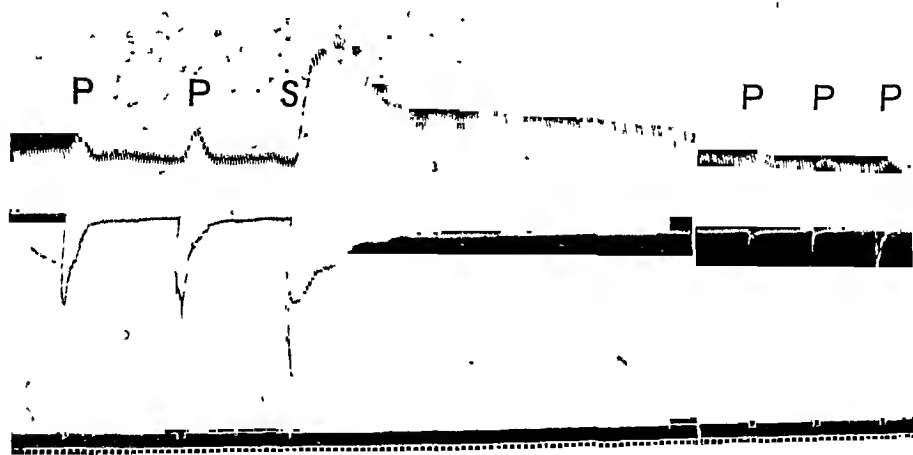


Fig. 9 $\times \frac{1}{2}$. Cat. Record of blood pressure (upper curve) and of contraction of the right upper abdominal rectus. *p* = response to pinching the pancreas, before and after dividing the right splanchnic nerve at *s*. The responses are much diminished by the splanchnic section. A piece of curve of 10 min. duration has been excised.

duodenal loop (Fig. 8 and 9); what subsequently remains of response, and the response from the tail of the pancreas, is abolished or almost abolished by section of the left splanchnic nerve. Thus it is clear that we have to deal, so far as abdominal wall reflex is concerned, with a reflex conveyed through the anatomical sympathetic nervous system.

Sometimes each response of the abdominal muscle to pinching the pancreas is accompanied by movements of the right leg, or of both legs; these vary; there may be kicks or the trotting movement of the hind legs may appear, but the predominant movement is stretching with extension.

Finally, emphasis is to be laid on the similarity of responses from pancreas and from back muscles. If pancreas and back muscles of the right side are pinched alternately, records from the right rectus are obtained (Fig. 10) in which the two responses can be compared with accuracy. The responses are exactly the same whether derived from the somatic muscles

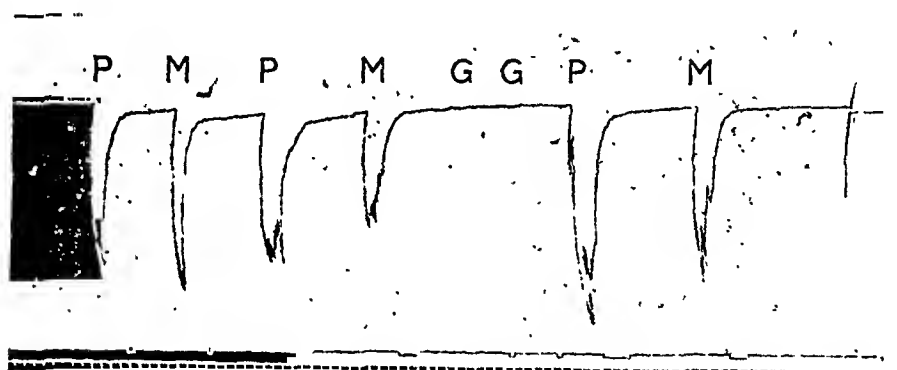


Fig. 10 \times 1. Cat. Record of contraction of right upper abdominal rectus. A series comparing result of stimulating pancreas and back muscles. P = responses to pinching pancreas. M = responses to pinching back muscles at level of 12th thoracic spine. G = no response to pinch of gut wall, causing strong contraction.

or from the viscus, and conveyed in the latter case along sympathetic pathways. Likewise the leg responses to visceral stimulation and to stimulation of body wall musculature though variable in type are alike in any given animal.

Blood pressure.

A rise of blood pressure is the rule as a response to cutting the skin of the abdominal wall; there is a larger response to pinching the back muscles; a still larger and the most prolonged response occurs when the pancreas is pinched, though this rarely amounts to more than 15 mm. Hg. The pancreas stimulus more effectively raises pressure even when this stimulus

and pinching the back muscles give equal responses of the rectus muscle (Fig. 11); the first rise is usually followed by a less distinct second rise when the pancreas is pinched; similar double peaks of pressure are seen in Sherrington's curves (22) when the response is to stimulation of the bile duct. A rise of blood pressure, of extent similar to that from pancreatic stimulation, occurs when the bowel itself is pinched although the abdominal wall shows no response by contraction (Fig. 12). Thus, the afferent impulses responsible for the vasopressor reflex are manifestly distinct from those starting the visceromotor reflex.

GENERAL DISCUSSION.

The foregoing observations, derived from distinct sources of experiment, are all relevant to the long controversy which has concerned the manner in which visceral pain and the associated tenderness and muscular rigidity arise and are referred.

The controversy relating to pain of visceral origin has depended largely upon the survival of the hypothesis advocated by Ross (19) and by Mackenzie (13). This hypothesis was introduced by Sturge (23) in 1883, when he said that the radiation of pain in angina is an evidence of an extension of commotion from one small patch of grey nerve substance to other parts of grey matter more or less intimately associated with it; and that soreness and tenderness left by the attack are due to the centre remaining in an irritable condition, causing it to overact to ordinary stimuli. Though Sturge had no substantial support to bring for this hypothesis, Ross was fascinated by it and used it to explain what he called the somatic pains of visceral disease in general. He believed that pain may be derived directly from a viscus and he called this *splanchnic* pain. He believed that a second pain is derived indirectly from the viscus and called this *somatic* pain. "When the splanchnic peripheral terminations of these nerves are irritated the irritation is conducted to the posterior roots of the nerves, and on reaching the grey matter of the posterior horns it diffuses to the roots of the corresponding somatic nerves and thus causes an associated pain in the territory of distribution of these nerves, which may appropriately be named the somatic pain." Lennander's work (8) followed. Mackenzie studied this and was quite convinced that the viscera are totally insensitive. Consequently he refused Ross's belief that pain impulses are conveyed directly from the viscus (Ross's splanchnic pain) and, proceeding farther than Ross, explained all visceral pain as the result of irritation by non-painful impulses ascending from the viscus to the cord and spreading in it. Here is the full hypothesis, which was advocated so persistently that it came to receive wide acceptance in the early years of this century. But it has never received universal acceptance; for there have always been those who have remained convinced that there are two kinds of pain, one of which is a true visceral pain (Hurst (4), Ryle (20), Morley (16)), and there have been some who have refused the idea of an irritable centre in the cord.

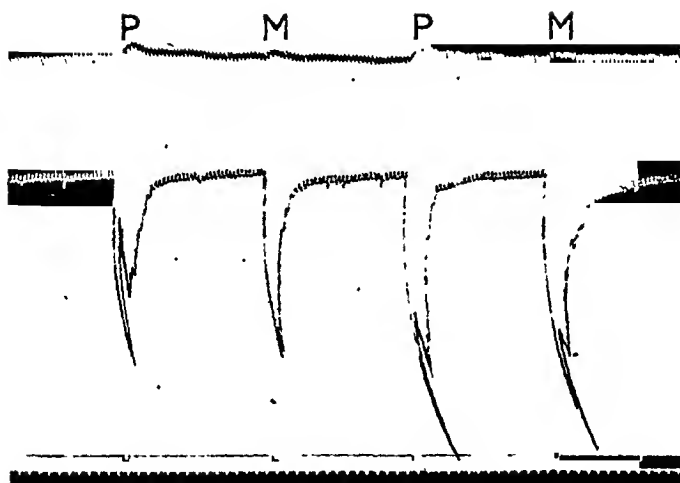


Fig. 11 $\times \frac{1}{2}$. Cat. Record of blood pressure and of contraction of right upper abdominal rectus. P = responses to pinching pancreas, M to pinching spinal muscle at level of 1st lumbar vertebra.

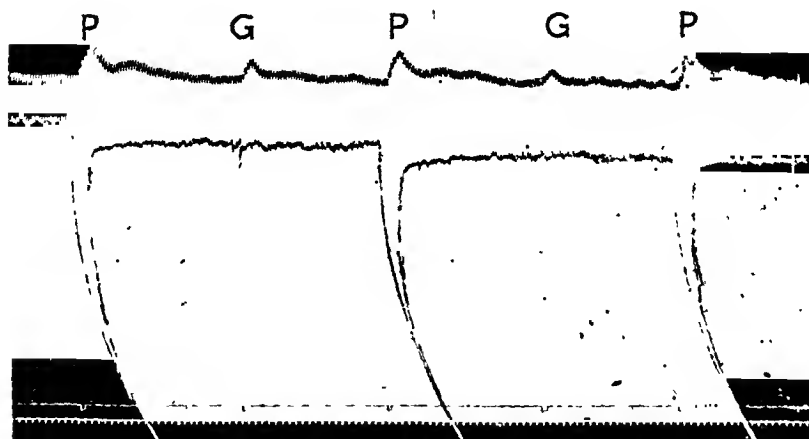


Fig. 12 $\times \frac{1}{2}$. Cat. Record of blood pressure and of contraction of right upper abdominal rectus. P = response to pinching pancreas, G to pinching duodenum, each pinch giving full and sustained contraction of the bowel wall.

The starting point of visceral pain. Let us come from this brief historical survey of hypothesis to a survey of fact and first ask if pain impulses are actually derived directly from the abdominal viscera? In our view, the most remarkable work that has been published in relation to visceral pain is that of Lennander (8), notable as it was both for precision and for wealth of observation. The insensitivity of the abdominal viscera had long been suggested by other surgeons. Lennander, who records their view, proved that the solid organs, and the main hollow organs, can be cut, burned, crushed, or stretched without the subject being in the least conscious of the injury. This work has been confirmed in its broad outline by all who have since made similar observations upon man. Lennander came to the conclusion for the human subject that the viscera named by him are incapable of giving rise directly to pain. It has been urged that special organs respond only to special stimuli—the eye to light, the ear to sound, and so forth—and that cutting and burning being unnatural forms of stimulus to apply to such a viscus as the bowel, may be incapable of initiating pain, but that pain may be derived directly from the bowel by the development of tension in its walls. Such an argument cannot pass. The test is not of an end-organ like the retina or a touch corpuscle, it is of nerve fibres. All nerve fibres that have been tested respond appropriately to cross section, the optic nerve when cut gives flashes of light, a motor nerve gives motor response, pain nerves give pain. It is inconceivable that viscera are permeated by pain nerves, and that these cannot be stimulated by similar means. It is difficult therefore not to accept Lennander's conclusion as Mackenzie did. Yet we know from common and special experience that contraction of the bowel can awaken pain. Mackenzie watched intestine lying outside an abdominal incision and at its visible contraction the conscious subject complained of colic. It was because he believed there are no pain nerves in the gut, and yet that pain can be started up by intestinal contraction that he was driven to accept the hypothesis of irritation of the spinal cord by non-painful afferent stimuli. There is, however, another way out, clearly pointed to by Lennander and others. This observer had also seen the bowel contract, even to obliteration of its lumen, but his statement is that such contraction does not necessarily cause pain (Bier (1) had said the same thing previously); and that pain comes only when so much gut contracts that it may be supposed to put tension on its mesentery. He found the mesentery, but not the gut, sensitive to tension in man, stretching one or other with his fingers. According to this view, pain comes in intestinal contraction, not because their walls pass into a state of contraction or of tension, but because they press or pull on structures to which pain nerves run.

Our own observations bear only indirectly on this point, unless it may be held that the chief afferent impulses ascending from the bowel are of one kind, ascending to the higher centres and giving pain, while side-tracked in the cord to give reflex motor responses in the abdominal wall. While we would

say that this is quite probable, it is not at the moment capable of proof, and so we shall not over stress in this particular phase of the discussion our finding that the afferent impulses, which awaken motor responses, can be provoked from the mesenteries and not from the guts themselves.*

Visceral pain as an entity. The second matter to be discussed is visceral pain as a special entity. Mackenzie certainly regarded it as provoked through a highly special mechanism; many who have followed him appear to have agreed at least so far as to regard pain of visceral origin as in a category of its own. We believe this concept to be wrong. Morley has already uttered the clear warning that in abdominal disease pain referred to distant points may be derived not from visceral but from somatic structures; he has insistently and rightly stressed shoulder pain arising from irritation of the diaphragm. It is an old illustration but one the full significance of which Morley has been the first fully to appreciate.† Our own observations lead in the same direction and amplify this point of view. The observations recently recorded by one of us on the reference of pain from the interspinous ligaments, and the observations here recorded on reflex muscular rigidities and areas of hyperalgesia, bring perfectly clear evidence that the peripheral reactions encountered in painful visceral disease, namely, pain distributed along segmental paths, appropriate muscular rigidities and tenderness of skin and body wall may all be derived from somatic structures lying within the corresponding segments; neither the referred pain, nor what have been termed the visceromotor and viscerosensory reflexes are hallmarks of visceral disturbance, they are all equally consistent with purely somatic disturbance. In man it has been shown that all the essential parts of the condition known as renal colic, pain diffused from loin to scrotum, iliac and testicular tenderness, iliac rigidity and cremasteric retraction, can be provoked by a stimulus confined to somatic structures, such as muscle or ligament of the spine. This is, therefore, not a picture peculiar to, though admittedly it is usually the result of, visceral disease. Similarly the essential features of the anginal syndrome, pain diffused to the elbow or even to the extremity of the upper limb, with peculiar feelings in the fingers, a sense of tension in the chest, of respiratory

* McDowall (14) found that pupillary dilatation may be obtained from the gut. He, and Irving, McSwiney and Suffolk (5), who have extended his observations, state that whereas in chloralosed cats cutting into the gut gives no dilatation, stretching the gut does. The manner of stretching is not stated in either case, and it may be that the effect described was derived from mesentery rather than from gut. In observations upon chloralosed cats we have found that a large pupillary reaction is readily provoked from the pancreas, when a reaction from the bowel itself is indeterminate or absent, in response to crushing the bowel or to distending a balloon within it. There is no doubt that the pupillary reaction to mesenteric stimulation is relatively very conspicuous and constant. We are unable to conclude from our own observations or from previous records that in circumstances in which a pinch or cut of the bowel gives no pupillary reaction, balloon distension is any more successful; the reaction from the balloon distension never seems to be large and, on occasion we have seen equal reactions from pinching the bowel.

† While we are here in sympathy with Morley we cannot always accept his view of the manner in which referred phenomena arise in visceral disease, thinking it probable that they are derived more frequently from the posterior structures of the abdomen including the mesenteries than from the anterior or lateral walls.

embarrassment, and of unwillingness to inspire, can all be reproduced by similar stimulation of the appropriate somatic structure. These observations are fatal to the idea that any of the complex visceral syndrome is peculiar to primary visceral disturbance and they at once raise the more general question. Is there in fact any distinguishing feature of these syndromes, other than the manner in which they are provoked, which conclusively stamps them as visceral? We believe there is none; even the character of the pain derived from somatic and visceral sources, provided the former is deep seated, seems from our deliberate comparison to be of one kind. It is to be realised clinically that in the presence of a characteristic syndrome, such for example as is ordinarily the product of cardiac or renal disease, the phenomena of this syndrome are not necessarily derived from the viscus; they may be derived on occasion from sensitive somatic tissues of the segment or segments concerned.

Although the discovery, that all the chief subjective phenomena usually associated with visceral disease can be reproduced through purely somatic channels, is opposed to the concept that somatic and visceral pain are to be regarded as separate entities, it does not preclude the idea that pain in visceral disease may be derived either from visceral or from somatic structure. Mackenzie's view was that in visceral disease all sensory and motor reflexes are derived from afferent visceral impulses. Morley's view conflicts with this, for he believes that all the referred phenomena in abdominal disease are derived from the parietes and none directly from afferent impulses ascending from the viscus. Mackenzie was certainly wrong in regarding the viscus itself as the exclusive source of the afferent impulses concerned. It is also impossible to accept in its integrity Morley's thesis, which is inconsistent with our demonstration that motor reflexes may originate in stimulation applied to the pancreas and conveyed through the splanchnic nerves.

If the terms "visceral" and "somatic" pain were used to convey merely the idea that the pain impulses come on the one hand from a viscus or its supports, and on the other hand from the body wall, then there would seem to be no harm in their survival. But they are not used in this strictly limited sense, they are used in a manner to imply that the systems of nerves concerned are distinct physiologically, that one or other system is alone capable of displaying referred phenomena, or that the mechanism of pain in the two cases is fundamentally different. The idea that a specific form of pain termed visceral, can be separated off on these lines is one for which no satisfactory evidence seems to us to remain; it is in our view an unnecessary distinction. Certain parts of the abdominal contents may be proved directly to possess pain nerves that respond to such stimuli as pinching, cutting, tearing or faradism. We can find no adequate reason to suppose that pain arising directly from the abdominal contents ever passes by any nerves

but these.* The onus of proof lies quite definitely with those who hold the different view that there is a special form of visceral pain conducted by afferent nerves of a different order.

Similarly there is no reason to distinguish in any fundamental way between nerves conveying pain from deep somatic structure or from any sensitive visceral structure. It is a matter of very little theoretical importance whether in the case of renal colic pain is derived from ureteric wall or from tissues closely surrounding the ureter. There is no advantage to be derived from dividing sensory nerve fibres according to their anatomical distribution, according to whether they run to the cord through the somatic nerves or pass first through the sympathetic chain.† It is known that nerves conducting pain impulses travel in both anatomical systems—thus, pain of cardiac origin may be blocked by extirpating sympathetic cervical ganglia (Sutton and Lueth‡ (24)), Richardson and White (18); the inferior cervical ganglia and the cardiac and the splanchnic nerves are painful to stimulation in man (Jonescu and Ionesco (6), Leriche (9))—and there is no evidence that the type of pain awakened, or the associated reflexes, differ in the two cases. It is impracticable thoroughly to compare pain derived through direct stimulation of the sensory nerves of the two anatomical systems in man, and it is impossible to do so in animals. But we can compare the associated phenomenon, the muscular reflex, derived from the two sources, in animals. This we have been at pains to do in some detail, and it shows us unequivocally that afferent impulses set up on the one hand from the duodenal loop and travelling by the splanchnic nerve, and on the other hand from the tissues of the back, provoke muscular reflexes that are indistinguishable in character. Thus it is proved that afferent impulses travelling respectively in sympathetic and somatic nerves can exert the same ultimate reflex effects. If these same impulses are transmitted to the sensorium and are interpreted there as pain, which is highly probable, then it is equally probable that the pain, like the reflex, will have a common pattern. To put the matter briefly and generally, we believe that the pain of visceral and somatic disease is derived from the direct stimulation of a common system of pain nerves, which supply all the deep lying somatic tissues concerned and, by way of the anatomical

* The problem with which we are dealing is a general one; the precise manner in which these nerves may be stimulated by disease of different organs is one that does not concern us in this paper. Thus, because we have referred especially to tension conveyed to mesenteries by contraction of a hollow viscus, we do not desire to convey the idea that this is the only form or place of stimulation; any form of stimulation that arises, and which is adequate to stimulate pain nerves in general, will suffice to stimulate those concerned.

† The terminology has often confused, and continues to confuse, discussion. The term sympathetic is used in two senses, it is applied to an anatomical system of nerves comprising the paravertebral chains and their connections; and it is applied to a physiological system of nerve fibres having cell stations in the sympathetic ganglia. When writers use the phrases "sympathetic sensory" or "sympathetic afferent" nerves, they may mean merely that sensory or afferent fibres run temporarily in the anatomical sympathetic to pass ultimately through the posterior roots. But the use of such expressions is apt to give the false idea that there is a special system of sensory or afferent nerves belonging exclusively to the physiological sympathetic system.

‡ These workers wrote of removing the *annulus viousiensis*, but correspondence with thönn shows that this removal involved the whole sympathetic pathway from the heart.

sympathetic, also supply a limited amount of the tissues contained within the visceral peritoneum; and that these pain impulses are either identical with, or are generally associated with, the afferent impulses which set up reflexly a common series of motor and sensory reactions. It is largely a matter of indifference whether the nerve fibres stimulated arise from visceral or deep lying somatic tissue, it is a matter of indifference whether they pass to the posterior roots by way of an anatomical path grouped as somatic or sympathetic; the result will depend (apart from strength and duration of stimulus) chiefly upon the segmental derivation of the afferent fibres concerned.

This relatively simple generalisation carries with it the practical conclusion that the fundamental mechanism (or mechanisms) underlying pain and associated reflexes, being common to both somatic and visceral disturbance, may be studied in either. So long as pain of visceral origin is to be regarded as fundamentally peculiar, then it would seem incapable of thorough experimental investigation owing to the inaccessibility of the tissues concerned in man; but if it differs only in the source from which it is derived, then the main problems can be thoroughly probed in accessible somatic tissues, for they are problems concerning the general mechanism of pain derived from any deep lying tissue.

Skin hyperalgesia. In concluding that there is but one system of afferent nerves underlying pain of deep origin with the associated reflex phenomena deep tenderness and muscular rigidity, we reserve for special consideration reflex hyperalgesia of the skin. It is very important that cutaneous and deep tenderness should be regarded separately. They are clearly different phenomena; their segmental areas do not correspond, and they develop and subside differently. In patients, used to angular pain with skin tenderness, this tenderness and that provoked by injecting the back appear to be indistinguishable both in place and kind; both are described as lesser or greater soreness of the skin to light friction, sometimes, but unusually, amounting to discomfort from friction of clothing. They both last for one or many hours. Thus, they resemble each other closely enough to be regarded as arising in a common fashion.

The mechanism of skin tenderness referred from a distant point of skin stimulation has been studied extensively by one of us (10), and has been thought to arise by axon reflexes* through what have been called "nocifensor" nerves. The belief here expressed that the tenderness arising from visceral disease and from stimulation of the back are identical does not hang upon the nocifensor theory, which should be considered on its own separate merits; but we naturally attempt to bring all together. It is quite clear that the type of referred skin tenderness provoked by stimulation of skin itself or by stimulation of deeper lying structures is of the same kind; thus the example of stimulating the interior of the maxillary antrum has been

* If they have been shown to occur through axon reflexes, that does not exclude the possibility of the longer excursion, which a cord reflex would provide, on occasion.

given in a previous paper. In the present observations we have used stimulation of ligaments of the back, all three (skin, antrum, ligament) yield similar hyperalgesia, and hyperalgesia that develops or continues to develop, both in intensity and in extent, after painful stimulation has ceased and customarily lasts for one or many hours. In all cases too the extent of this hyperalgesia is limited by nerve or segmental territory. It is to be emphasised that these tests (present and past) have been made for the most part on the same individuals, thus rendering comparison more exact. Thus we find strong evidence that the hyperalgesia comes in each case through a single mechanism. In this connection it is to be said that Morley (17) relates that in patients in whom he stimulated the diaphragm, hyperalgesia appeared instantly at the shoulder tip and vanished at once when stimulation ceased. For several reasons this example cannot be regarded as establishing a fundamental distinction. Firstly, it can hardly be doubted that cutaneous hyperalgesia derived from ligaments of spine and of diaphragm are similarly brought about, for both are derived from deep lying somatic structures, and the one serves as well as the other as example; yet in the case of back muscles we have found the hyperalgesia to take some time to develop and much time to subside. Secondly, Capps and Coleman (2) in similar tests of the diaphragm found hyperalgesia distinctly to outlast the pain.* Thus, there is disagreement about the duration of hyperalgesia provoked by stimulating different deep lying somatic structures. Perhaps it will prove more accurate to say that the duration of hyperalgesia varies from subject to subject. In the special studies of skin tenderness previously published from this laboratory variation in the effects from subject to subject has been stressed, but in the long argument upon mechanism this may be forgotten. The fact is that stimulating neighbouring skin in some subjects fails to provoke cutaneous hyperalgesia; in some too itching appears instead. Moreover, when the hyperalgesia appears, in some it lasts many hours, in others it lasts for shorter periods. Goldscheider (3) described it as disappearing instantly with the pain. Thus, there is as much discord in the description of what is found after skin stimulation as after stimulating deeper lying structures; and the similar variations in the two instances become in reality a further point of resemblance between them. The presence of hyperalgesia of the skin in one patient with abdominal disease and its absence from another, despite pains of similar intensity and duration in both, is to be explained plausibly along the same lines.

There is a point of theoretical interest that does not affect this main argument. It has been thought by one of us that pain derived from the skin and from deeper lying structures may need to be regarded as separate, in that they seem qualitatively distinguishable and are apt to awaken different forms of reflex (11). The reflex previously stressed was the vasovagal.

* It is not always made abundantly clear in such experiments that skin tenderness and deep tenderness are distinguished; the distinction is from our standpoint important, as we believe skin tenderness and this only to be a phenomenon that may be delayed in onset and long outlasting the stimulus.

This view of separate pain systems is strengthened by the motor reflexes observed in the present work. Painful stimulation of the skin or nerves to the skin in the cat provokes only kicking movements of the legs, stimulation of all sentient deep structures provokes reflex contraction of the abdominal muscles and usually distinct movements of the legs, like stretching. If we believed hyperalgesia to depend upon axon reflexes passing through pain nerves, it would be difficult to explain how the impulse became transmitted from the deep system of pain nerves to a distinct system supplying the skin; but so long as the underlying change producing hyperalgesia is thought to depend on reflexes travelling in a common system of nocifensor fibres, distinct from pain nerves, this theoretical difficulty does not arise.

To sum up, if the view that cutaneous hyperalgesia resulting from skin stimulation is brought about through nocifensor nerves is accepted, then it may be held that cutaneous hyperalgesia brought about reflexly from any source is similarly caused.

SUMMARY AND CONCLUSIONS.

1. Stimulation of an interspinous ligament in man gives pain, superficial tenderness, deep tenderness, and tonic contractions of muscles of the trunk having appropriate segmental distributions. The phenomena thus grouped are similar to, or identical with, those associated with visceral disease in man, and customarily described as "referred."

2. By stimulating appropriate somatic structures it is possible to provoke pains and accompanying subjective sensations that are indistinguishable in quality and similar in distribution to those of angina of effort or intestinal colic.

3. In the decapitated cat reflex muscular contractions of the abdominal wall can be provoked either by stimulating the spinal muscles, or by stimulating such a viscus as the pancreas; the contractions are indistinguishable, but the afferent path for the first is somatic, and that for the second passes through the sympathetic (splanchnic) nerves.

4. In the decapitated cat, visceromotor reflexes are regularly elicited by pinching or otherwise stimulating structures (pancreas, etc.) lying in the mesentery of the duodenal loop, but no such reflex can be provoked from the bowel itself by injury, by distension, or by causing contraction. All these stimuli, however, yield a rise of blood-pressure.

5. In the decapitated cat injury of the skin yields energetic kicking movements, but no reflex movements of abdominal muscles; injury of the lower muscles of the back or of the abdominal wall yield vigorous reflex contractions of the abdominal wall and smaller leg movement that are usually of different type.

6. We conclude that there is no special form of pain, referred or otherwise, and no special viscerosensory or visceromotor reflex, which is the hallmark of visceral disease. Pain of visceral and of somatic origin cannot

be distinguished as such. Deep somatic and certain visceral structures are supplied by a common set of afferent nerves (including pain nerves), stimulation of which produces similar pain and many similar reflex phenomena; this common system is responsible for all the pain and referred phenomena of visceral disease. Variations in reaction depend chiefly upon strength and duration of stimulus and upon the segmental derivation of the afferent fibres stimulated.

7. Vascular and motor reflexes from visceral structures seem to arise separately.

8. Cutaneous hyperalgesia appears to be of the same kind whether provoked by visceral disease, by stimulating deep somatic structures, or by stimulating neighbouring skin. We suggest, though we cannot show, that in each case hyperalgesia is provoked through "nocifensor" nerves.

9. Reasons have been brought forward for the view that the pain nerves of the skin belong to a system separate from those running to deep lying structures. The difference in the reflexes associated with stimulation of afferent paths from skin and deep structures lends additional support to this view.

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A NOTE ON THE SERUM SODIUM LEVEL IN PATIENTS SUFFERING FROM TUBERCULOSIS.*

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PATIENTS suffering from tuberculosis not infrequently show signs and symptoms of adrenal damage, although the full classical picture of Addison's disease is rarely present. Moreover, it is known that the serum sodium level falls, both in man and in most animals, in adrenal cortical insufficiency, (4, 5, 6, 7, 9, 10, 11). Therefore we thought it might be of interest to find the sodium content of the blood serum in tuberculous patients and to try to correlate the results with the clinical condition and prognosis.

The observations were carried out on 169 consecutive admissions to Glenlomond Sanatorium. Pulmonary tuberculosis was the most obvious feature in the majority of the patients. On clinical grounds alone they were divided into four groups. Group I was composed of 59 patients who had made a clinical recovery, group II of 42 patients who were moderately well, group III of 36 patients seriously ill, and group IV of 32 patients who died from tuberculosis during the course of the investigation.

When an autopsy was obtained, one adrenal was examined histologically by serial section, and a portion of the other adrenal macerated and injected into guinea pigs.

Sodium balances were carried out by placing seven patients on a standard diet for five days. After a preliminary period of two days, sodium was estimated in the food, in the urine, and in the faeces during the subsequent three days. No attempt was made to estimate the sodium lost in sweat. Blood was taken on the morning of the last day.

In all cases blood was drawn for sodium estimation early in the morning before breakfast, care being taken to avoid contamination with sodium salts. For blood serum and for urine and uranyl-zinc acetate gravimetric method of Butler and Tuthill (2) was used, for faeces the modification devised by Stiven (15). In many of the patients belonging to groups III and IV several estimations of the serum sodium were made.

* We are deeply indebted to Dr. W. T. Munro, Superintendent of the Sanatorium, for his kindness and for the facilities he placed at our disposal.

TABLE I.

Actual and percentage distribution of the patients in the four clinical groups according to the serum sodium level.

Group of patients.	Serum sodium in mg. per 100 c.c.						
	275-284	285-294	295-304	305-314	315-324	325-334	335-344
I. 59 recovered.				5 8.5%	24 40.7%	23 39.0%	7 11.9%
II. 42 moderately well.			3 7.1%	6 14.3%	18 42.9%	12 28.6%	3 7.1%
III. 36 patients ill.			6 16.7%	8 22.2%	15 41.7%	7 19.4%	
IV. 32 patients dead.	2 6.3%	4 12.5%	13 40.6%	8 25.0%	5 15.6%		

TABLE II.

Signs and symptoms with reference to serum sodium.

Signs and symptoms.	Sodium per 100 c.c. serum.	
	55 patients below 315 mg.	114 patients above 315 mg.
Slight pigmentation.	8 (15%)	None.
S.B.P. below 100 mm. Hg.	26 (47%)	9 (8%)
Diarrhœa.	11 (20%)	7 (6%)
Vomiting.	7 (13%)	5 (4%)
Much pyrexia.	34 (62%)	18 (16%)
Pleural effusion.	4 (7%)	6 (5%)
Myasthenia.	42 (76%)	34 (30%)

TABLE III.

Post mortem findings in tuberculous patients.

Post-mortem findings.	Sodium level per 100 c.c. serum.	
	21 patients below 315 mg.	3 patients above 315 mg.
T.B. found by inoculation of adrenal tissue into guinea pigs.	6	none.
Histological evidence only of damage to adrenals.	3	none.
Amyloid degeneration of adrenals.	1	none.

TABLE IV.

Results of three-day balance experiments.

Patient.	Serum Na. mg. per 100 c.c.	Sodium mg. average daily.		
		Intake.	Loss by kidneys.	Loss in faeces.
G.D.	298	2.5	0.9	0.03
W.J.	309	3.2	1.7	0.04
J.D.	311	3.0	2.5	0.04
O.M.	315	5.0	3.1	0.02
G.M.	323	5.0	3.9	0.03
J.T.	325	5.0	3.8	0.03
W.F.	333	5.0	2.5	0.08

Results.

The average sodium level in healthy individuals is said to be 330 mg. per 100 c.c. serum (1, 3, 8, 11, 14). Thus, a division at 315 mg. should, unless in very exceptional circumstances, separate the normal from the abnormally low. On this basis, 114 of our patients had a "normal" serum sodium, 55 a "low" value.

Much more striking and significant is the distribution of the values within the groups. This is shown in Table I. There is an obvious shift to the left as the condition deteriorates.

Table II shows the incidence of signs and symptoms with reference to the serum sodium level, Table III gives the post mortem findings in similar fashion, and Table IV summarizes the results of the balance experiments.

Discussion.

The fundamental work of McCance (11, 12) shows that the normal human body responds at once to decreased sodium intake by diminished excretion of sodium. Even in the face of an acute alkalæmia the body will not yield its base (13). Very profuse sweating, combined with copious drinking of water, must be superimposed on a sodium-free diet in order to lower the serum sodium level to the extent found in many of our patients.

It is known, however, that the level may be lowered by acute conditions such as severe diarrhoea and pyloric stenosis or intestinal obstruction with vomiting. Although some of the patients in the present series inevitably suffered to a greater or lesser extent from anorexia, sweating, diarrhoea or vomiting, yet these signs and symptoms were by no means confined to the individuals with a low serum sodium level (Table II). None of them, even with the greatest distaste for food, had a sodium intake at all comparable to the low level used by McCance in his experiments. Yet, in many cases, the serum sodium was persistently subnormal, and, as the balance experiments suggest, they were still losing sodium by the kidneys. This is just what happens in frank cases of Addison's disease (16). In normal people one would expect renal loss of sodium to cease under such conditions. It does seem justifiable to suspect that the control of sodium metabolism was deranged in many of our patients.

Unequivocal proof of injury to the adrenal cortex was found in 9 of 24 cases dying of tuberculosis and coming to autopsy. There was one case of amyloid degeneration. All these patients were in the "low" sodium level group. This incidence of adrenal damage is high, indeed much higher than the figures given in past records, but, of course, the series is small.

It may be that any prolonged toxic condition with prostration and asthenia is accompanied by a low level of serum sodium without any specific involvement of the adrenal cortex. Tuberculosis is, however, a general infection. The adrenal cortex may conceivably be affected, either by actual organismal invasion or by toxin, at a much earlier stage of the disease than hitherto suspected.

SUMMARY.

1. The serum sodium was estimated in 169 patients suffering from tuberculosis, and was found in most instances to be lower than is usually accepted as normal.

2. In general the poorer the clinical condition, the lower the serum sodium.

3. Simple deficiency of sodium intake did not seem to be capable of explaining the low levels found.

4. It is suggested that derangement of the adrenal cortex was responsible, in part at least, for the findings.

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OCULAR EFFECTS OF SYMPATHETIC STIMULATION IN MAN.

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THE effects of cervical sympathetic stimulation on the eye in animals have often been investigated since Claude Bernard published his paper (3) in 1852, but the corresponding phenomena in man have been less examined. The present paper is concerned chiefly with the rôle of the ocular sympathetic in man, but also with certain results obtained in animals.

Sympathetic stimulation in experimental animals.

When the distal end of the cut cervical sympathetic is stimulated electrically, there may be widening of the pupil and palpebral fissure, retraction of the nictitating membrane, and protrusion of the globe. Three points will be considered, namely, widening of the palpebral fissure, protrusion of the globe, and the thresholds of stimulation required to produce the separate phenomena.

Palpebral fissure. Widening of the palpebral fissure may be due in part to the development of exophthalmos, but is chiefly due to retraction of the lids by the smooth superior and inferior tarsal muscles, which are inserted into them (30). The contribution of the two lids differs in different species. In the dog (8) and the rabbit (35) it results from a lowering of the lower lid, the upper lid being unchanged in position; Poos correlates his result in the rabbit with the poor development of the superior tarsal muscle in this species. In the urethanised cat, Velhagen (45) found that both lids retracted; I have found the main effect here to be a lowering of the lower lid, but the upper lid may be slightly raised, particularly in its anterior part. In apes, however, Hesser (16) found conspicuous elevation of the upper lid, and was doubtful whether the lower lid moved. In man the major effect is on the upper lid, but the lower lid also participates (*see* p. 83).

Exophthalmos. It is extremely difficult to determine by naked eye methods whether exophthalmos occurs if retraction of the lids is developing

* Work undertaken on behalf of the Medical Research Council.

simultaneously. An illusion of considerable proptosis results from the retraction of the lids and nictitating membrane with mydriasis, even though unassociated with actual proptosis. Therefore, as has often been emphasised, it is difficult to accept reports of exophthalmos unless it has been detected instrumentally. It is quite clear, however, that stimulation of the sympathetic in anaesthetised animals *may* result in exophthalmos. For example, Essex and Corwin (9) report that in the dog, anaesthetised with pentothal-sodium the globe is protruded by 5 mm. on sympathetic stimulation, and exophthalmos has also been measured in the cat (6, 25) and rabbit (17, 25) but repeatedly denied in apes (2, 25, 29). However, recent work of Essex and Corwin (9) makes it necessary to reconsider both the significance of this exophthalmos and the experimental conditions under which it is sought. These workers found that in dogs certain anaesthetics cause an enophthalmos, and that the eye is then brought back to its normal position by sympathetic stimulation, tyramine or ephedrine; and that this advance of the eye is not obtained on stimulation after anaesthetics which do not cause a preliminary enophthalmos.*

I have found that stimulation of the cut sympathetic produces consistent but slight ($\frac{1}{2}$ to 1 mm.) exophthalmos in the cat under urethane, and very little or no exophthalmos in the rabbit under urethane or nembutal. I have not investigated if exophthalmos from sympathetic stimulation in the cat resembles that in the dog in being merely a restitution of the normal position of the eye, the unanaesthetised cat being less suited than the dog, both anatomically and temperamentally, to measurements of exophthalmos.

Thresholds. In four cats I have investigated the conditions necessary for obtaining constant thresholds for sympathetic stimulation. The cat is anaesthetised with 4 c.c. of 25% urethane per kilo injected subcutaneously, and an equal quantity intraperitoneally half an hour later. The sympathetic chain, about 2 cm. below the superior cervical ganglion, is ligated and cut after being freed from the vagus trunk which is tested electrically for ocular sympathetic fibres. Ligation stimulates transiently. The distal end is lightly placed over a pair of platinum electrodes and the ligature wound a few times between and round the points of the electrodes but without crossing or stretching the nerve. The electrodes are mounted on the table so as to exert no traction on the nerve. A small pledget of cotton wool is placed over the nerve on the electrodes, and saline allowed to drip continuously onto one end of it, so as to keep the other end covering the nerve constantly wet. Under these conditions the thresholds to faradic stimulation of the various ocular phenomena remain constant.

* It is important to realise exactly what Essex and Corwin have reported. They showed that pentothal produces enophthalmos, and that ether does not; and that stimulation after section of the sympathetic results in both cases in a normal position of the eye. They do not state, however, whether sympathetic section under ether results in an enophthalmos which is then abolished by sympathetic stimulation; or whether sympathectomy is associated with enophthalmos after recovery from the anaesthetic.

TABLE I.

*Thresholds in cm. for sympathetic stimulation.**Animals under urethane.*

	LEFT SIDE.	RIGHT SIDE.
Cat 1.	8 observations.	
Upper lid	About 8.	
Lower lid	$11\frac{1}{4}$ — $12\frac{1}{4}$ (av. $11\frac{1}{2}$)	
Nictitating memb.	$11\frac{1}{4}$ — $12\frac{1}{4}$ (av. 12)	
Pupil	$12\frac{1}{2}$ — $13\frac{1}{2}$ (av. $13\frac{1}{2}$)	
Exophthalmos	11 — $11\frac{3}{4}$ (av. $11\frac{1}{2}$)	
Cat 2.	10 observations.	15 observations.
Upper lid	$10\frac{3}{4}$ — $11\frac{1}{2}$ (av. $11\frac{1}{4}$)	9 — $10\frac{3}{4}$ (av. 10)
Lower lid	$11\frac{1}{4}$ — $12\frac{1}{2}$ (av. 12)	10 — 11 (av. $10\frac{1}{2}$)
Nictitating memb.	$11\frac{1}{2}$ — $12\frac{1}{2}$ (av. 12)	10 — 11 (av. $10\frac{1}{2}$)
Pupil	$11\frac{1}{4}$ — $12\frac{1}{2}$ (av. 12)	$10\frac{1}{2}$ —11 (av. $10\frac{3}{4}$)
Exophthalmos	$11\frac{1}{4}$ — $12\frac{1}{4}$ (av. 12)	10 — 11 (av. $10\frac{1}{2}$)
Rabbit 1.	6 observations.	2 observations.
Lower lid	7 — $8\frac{1}{2}$ (av. $7\frac{3}{4}$)	8, $7\frac{3}{4}$
Pupil	$6\frac{1}{2}$ — $8\frac{1}{2}$ (av. $7\frac{1}{2}$)	8, $7\frac{1}{4}$
Rabbit 2.	6 observations.	
Lower lid	13 — 15 (av. $13\frac{1}{2}$)	
Pupil	$13\frac{1}{2}$ — $15\frac{1}{2}$ (av. 14)	

In determining thresholds, faradic stimulation is applied for 10 sec. every minute, starting with the secondary coil in a sub-threshold position and advancing it $\frac{1}{4}$ cm. at each stimulation. Probably this routine is unnecessarily cautious, since I have never obtained any "facilitation" of a threshold stimulus by a strong stimulus shortly preceding it, nor any ocular response starting after 2 sec. of stimulation. Widening of the pupil and movement of the lids or nictitating membrane were observed by naked eye, confirmed if necessary by comparison with calipers set for the initial size. Actually, great precision is unimportant here since the full effects occur only a little above the threshold for just detectable effects. Exophthalmos was observed by using a light straw pointer, fixed at one end to the shaved skin of the bridge of the nose, hinged between nose and eye by being partly snapped, and held in contact with the apex of the cornea by the adhesion of a small point of seccotine. The free end beyond the eye moved across a scale with an exaggeration of about five times the excursion of the eye and, during adequate stimulation, moved sharply through 3 to 5 mm., and back after stimuli were discontinued. The same result was obtained in one experiment when both lids were retracted mechanically before stimulation. Ordinarily one observer watched for exophthalmos while another recorded the remaining effects. The phenomena studied had a latency of from $\frac{1}{2}$ sec., to about 2 sec. with threshold stimuli; they reached their full development after 2 to 3 sec. under maximal stimulation, and had returned almost to normal

15 sec. after its cessation. The threshold values remained constant over the periods investigated of 1 to 3 hours for each side (Table I). The actual values presumably depend on the dimensions of the electrode and the shunting resistance of the wet wool, and vary greatly if the electrode is reapplied for each stimulus. Similarly, slight drying and tension of the nerve decrease the values, either through hyperexcitability of the nerve or merely by altering the physical conditions at the electrodes.

The results also show that the various ocular effects of sympathetic stimulation occur with approximately equal thresholds. Their sequence has not been constant in different cats, mydriasis, for example, having once had the highest and once the lowest threshold. Poos (35) has stated that in the rabbit the threshold for mydriasis is lower than for lid retraction, while Brunton (6) quotes results on a chloralosed cat in which the mydriasis threshold was variable but lower than that for exophthalmos and higher than for retraction of the nictitating membrane and widening of the palpebral fissure. It is not clear whether these relations were constant in different experiments.

The rabbit appears to be less suitable than the cat for this work. Retraction of the upper lid and exophthalmos have been slight or absent, the response of the nictitating membrane has been variable, and the sympathetic may run in several cords with the vagus. The thresholds for mydriasis and retraction of the lower lid have been found to be approximately equal, and reproducible under the conditions described for the cat (Table I).

Electrical stimulation of the sympathetic in man.

In man there has been less accurate experimental investigation. In particular, the problem of exophthalmos is open to ambiguity; not only does the occurrence of exophthalmos in certain mammals prompt the assumption that it occurs in man, but widening of the palpebral fissure produces an illusory exophthalmos, and verification by measurement is infrequent. Faradic stimulation of the human cervical sympathetic has been reported on a number of occasions, either at operation under anaesthesia or, in two early cases, on heads after execution, by Müller (31,32) and by Wagner (48). All observers have seen mydriasis and a widening of the palpebral fissure, chiefly by elevation of the upper lid. Müller (31, 32) described and investigated the simultaneous retraction (downward) of the lower lid, and the participation of this lid is evident from the accounts of later observers (Unverricht (44), Jonnesco (24), Bailliart (1), and *see* p. 83). This is in conformity with the presence of smooth tarsal muscles in both upper and lower lids (30).

With regard to exophthalmos, statements conflict. In the earliest experiments Wagner (48) reported that no exophthalmos occurred, and Müller (32) said that there was no clear exophthalmos. In stimulation observed at operation, Jonnesco, (24), Reinhard (37), and Leriche (26) state that exophthalmos occurs, while Wölfflin (51), Chvostek (7) and MacCallum (29), state that it does not. All these authors apparently relied upon naked eye estimation of exophthalmos. Two observers, Unverricht

(44) and Mutch (33) have stimulated the sympathetic at operation while measuring the position of the eye by the Hertel exophthalmometer maintained *in situ*; both state that exophthalmos does not occur. I have confirmed this in the following cases.*

Case 1. In a woman of 28 years right cervical sympathectomy was done for Raynaud's disease, under nitrous oxide and ether. Faradic stimulation of the stellate ganglion (coil at 13 cm.) raised the upper lid from the closed position by about 4 mm., depressed the lower lid by about $1\frac{1}{2}$ mm. and produced mydriasis but no exophthalmos. In testing for exophthalmos the upper lid was held open so that the apex of the cornea could be watched throughout stimulation, using a more accurate modification (see p. 86) of Hertel's instrument, with which $\frac{1}{2}$ mm. of exophthalmos would have been readily detectable. This was repeated with the secondary coil at $9\frac{1}{2}$ cm., stimulating for 10 sec.; no exophthalmos developed.

In the same case during left sympathectomy 3 weeks later, similar results were obtained. Elevation of the upper lid and in this case only slight depression of the lower lid occurred, with mydriasis, but without any observed exophthalmos in observations in which 1 mm. of movement would have been detected. In this case pupil and lids reacted with the coil at 14 cm. and exophthalmos was sought with the coil at $7\frac{1}{2}$ cm. at which strength the pulse accelerated from 78 to 126. This tachycardia might conceivably be related to sensitisation by the previous sympathectomy.

Case 2. In a woman of 25 years right cervical sympathectomy was done for Raynaud's disease under nitrous oxide and ether. Strong stimulation of the stellate ganglion with the coil at 6 cm. gave mydriasis, elevation of the upper lid, slight depression of the lower lid (twice confirmed) but no exophthalmos (twice confirmed, to within $\frac{1}{2}$ mm.).

Case 3. In a woman of 30 years, left cervical sympathectomy was done for Raynaud's disease under nitrous oxide and ether. Mydriasis, elevation of the upper lid and depression of the lower lid by about $\frac{1}{2}$ mm. resulted repeatedly from stimulation of the stellate ganglion, but no exophthalmos was detected to within $\frac{1}{2}$ mm., even using strong stimulation with the secondary coil at 6 cm. which caused tachycardia and current spread to adjacent muscles.

In all these cases, the stellate ganglion was stimulated at a time when it was connected to the spinal cord by the 1st dorsal rami, but separated from the chain below it. All cases gave mydriasis, elevation of the upper lid and depression of the lower lid. Exophthalmos was never observed. In a further case stimulation of the chain below the stellate ganglion was found to have no effects upon the eye.†

* I am indebted to Mr. Gwynne Williams for enabling me to make these observations.

† It is of interest to note that after the operation all these cases had residual sympathetic paralysis on the side stimulated, although the first dorsal rami to the stellate ganglion were uncut. One case in which the ganglion was placed on the electrodes but not stimulated showed no such paralysis.

Since the only three observers, claiming to have obtained exophthalmos, relied on naked eye observation in the presence of confusing lid retraction, their statement of fact is difficult to accept. I have failed to obtain it with stimuli greatly above the threshold for lids and pupils, and strong enough to produce tachycardia, respiratory disturbance and current spread to neighbouring tissues; the corresponding thresholds in cats are approximately equal. It may be objected in the light of Essex and Corwin's work, that the anaesthetics employed may not induce the preliminary enophthalmos. It will, however, be shown later that in man section of the sympathetic does not cause enophthalmos (see p. 85).

Chemical stimulation of the sympathetic in man. It has long been known that cocaine applied to the conjunctiva produces mydriasis and widening of the palpebral fissure (20). The action is ascribed to stimulation of the sympathetic nerves and the effect is absent after their degeneration (33, 43). Poos (35) has suggested that the widening of the palpebral fissure might be ascribed to the anaesthetic action of cocaine in cutting out afferent stimuli which were causing some degree of orbicularis spasm or tone, but an effect is still obtained in cases of facial palsy (21) and the same phenomena are obtained with benzedrine (34), which has sympathetico-mimetic, but no anaesthetic properties. Jessop (22, 23) stated that occasionally after persistent cocaineisation in rabbits, and apparently from his account in man, exophthalmos might result, as judged by naked eye, but Bailliart (1) rejects this result. Birch-Hirschfeld (4) measured 0.4 mm. of proptosis after cocaine, which was the same amount as that produced by any equal widening of the palpebral fissure; Tuyl's measurements (42) confirm this.

I have instilled 2 drops of 2% cocaine solution into the conjunctival sac on one side in normal persons. Mydriasis occurred regularly, and widening of the palpebral fissure usually. Thus in 10 cases the upper lid was raised in 9, although only slightly in 2 of these, and the lower lid became depressed in 7. Exophthalmos of more than $\frac{1}{2}$ mm. has not resulted in any of these cases. I have obtained the same results with $\frac{1}{4}$ or 1% solutions of benzedrine. With each drug the upper lid has been raised from 1 to 2 mm. and the lower lid depressed about $\frac{1}{2}$ mm..

In most normal eyes, adrenaline solutions applied to the conjunctiva produce none of these effects. I have obtained mydriasis, without lid retraction, only on the affected side, using adrenaline (0.1%) bilaterally in 2 cases after resection of one stellate ganglion, and in one case of interruption of the sympathetic by a tumour in the orbital fissure (compare 19); but failed to obtain any effect in a case of unilateral sympathetic paralysis diagnosed as being due to a posterior superior cerebellar artery thrombosis.

It would be desirable to augment this account by clinical cases of irritative lesions of the sympathetic. Unequivocal examples of this type appear, however, to be extremely rare (46) or such at least as to produce signs other than mydriasis, which is said to be the earliest manifestation (5, 46).

Paralysis of the sympathetic in man.

It has been emphasised that widening of the palpebral fissure causes an illusion of exophthalmos. It is equally true that narrowing suggests enophthalmos. When Horner (18), described his original case of sympathetic paralysis in 1869 and observed ptosis and a small pupil on the affected side, he added that there was a very trivial degree of enophthalmos. Latterly, however, enophthalmos has come to be regarded as a clear and constant result of interruption of the sympathetic (Scarlett (40)). But when the position of the eye is measured instrumentally after surgical division of the sympathetic, it is found that it has not in fact receded, the appearance of enophthalmos being produced by the narrowed palpebral fissure. Only in a few cases and after a lapse of weeks or months slight enophthalmos may develop (28, 36, 43), presumably out of secondary changes in the orbit (47) rather than as a direct consequence of, for example, muscular denervation. This fact has been repeatedly confirmed by measurement (4, 28, 33, 39, 47), although denied by Weill and Nordman who made a personal communication to Bailliart (1) that 1 to 2 mm. of exophthalmos occurred regularly. No other details were quoted. Simple comparison of the two eyes after sympathectomy on one side are of course of little value in single cases, since the normal eyes may differ in position by at least 3 mm. relative to the orbital margins. The fullest investigation is that of Wagener (47) who compared the eyes before and after sympathectomy in 27 cases. The measurements were either unchanged or showed small differences in either direction within the range of error of the instrument used. In 94 cases of spontaneous Horner's syndrome he found an average enophthalmos of $\frac{1}{4}$ mm. comparing the two eyes.

Measurements in a few similar cases have agreed with these results. In seven cases of unilateral Horner's syndrome there has been no average difference in position between the eyes. In 3 cases of stellate ganglionectomy, measurements of the affected eye revealed only trivial changes after operation, namely, advancements by $\frac{1}{2}$ mm., 1 mm. and 1 mm.. In two patients, the sympathetic trunk was anaesthetised during the course of a phrenic avulsion under local anaesthesia. Horner's syndrome was produced and lasted about half an hour, measurements of the position of the eyes being obtained every few minutes before, during and after the sympathetic paralysis. In the first case, 15 readings before anaesthesia varied from $17\frac{1}{2}$ to $18\frac{3}{4}$ mm., 6 during anaesthesia from 18 to $18\frac{1}{2}$ mm. and 11 afterwards from $17\frac{1}{2}$ to $18\frac{1}{2}$. In the second case the 5 preliminary readings varied from $8\frac{1}{2}$ to 10 mm., 5 readings during anaesthesia from $8\frac{1}{2}$ to 10 mm., and subsequent readings of $8\frac{1}{2}$ and 9 mm. were obtained. It is clear that no enophthalmos results under these conditions in man. Myosis and the vascular results of sympathetic denervation will not be discussed. The narrowing of the palpebral fissure is the result of a descent of the upper lid ; and an ascent, although of less extent, of the lower lid, as has frequently

been observed and reported (11, 12, 33, 38, 43). In 15 cases I have regularly observed a descent of the upper lid by about $1\frac{1}{2}$ mm., and an ascent of the lower lid by about $\frac{1}{2}$ mm. which was doubtful or absent in 4 cases. I have seen no operated case in which the onset of Horner's syndrome was delayed for a period of hours after the section as has been described*, and in the anæsthetised cases the effects were maximal within a few minutes of the injection.

Functions of the tarsal muscles.

It is evident that the smooth tarsal muscles maintain some degree of tone in normal circumstances. However, it has not been proved if they participate in voluntary or associated movements of the lid. It is usually held that they do not do so (10, 13, 14) and the muscles themselves may be degenerate in old subjects (14). Wilbrand and Saenger (50) quote a case having cranial nerve lesions and complete ptosis of one upper lid, which could not be moved voluntarily but was raised slightly when the eyes looked upward. They regarded this as evidence that the smooth muscles participate in associated movements.

Some evidence on this point may be obtained by comparing the eyes in cases of unilateral sympathectomy. The difference between the upper lids in such cases does not increase with the lift of the lids as the eyes are raised nor is there any difference between the elevation of the two lids in staring.† It is concluded that the smooth muscles do not contribute to voluntary movements of the upper lid.

Measurement of exophthalmos.

The need for measurements of exophthalmos may arise in several rather different circumstances. Firstly, it may be desirable to decide whether both eyes are proptosed or are in a normal position. I shall not discuss here this awkward problem, which involves the choice of base-line, axis of measurement and limits to the normal. Secondly, one eye may be compared with its fellow as a test for its abnormality of position. This also I shall not consider except to confirm the previous findings of large differences in position between the normal eyes from whatever point they are measured, reaching 3 mm. or more in some cases.

Thirdly, however, it may be necessary to obtain serial measurements of the position of the eye, and this constitutes an entirely different problem, calling for a much higher precision than is necessary in the other cases, and necessitating modifications to Hertel's relatively inaccurate instrument (15) which is otherwise in wide use. At the same time the points from which the measurements are made may be chosen from convenience, and not for their constancy of relationship to the eye itself in different individuals. I have used an instrument‡ which rests on both inferior orbital margins and on the centre of the forehead. In an extended series of measurements it is desirable to exclude the effects of possible changes in the fat or other content of the tissues by periodic additional measurements to the lateral orbital margins where the skin and subcutaneous tissues are very thin.

* Leriche (27) has described a remarkable case in which a ptosis with complete closure of the denervated eye developed slowly during the weeks following operation; on the basis of this result, which was ascribed to the sympathectomy, he postulates an inhibitory action of the sympathetic on a tendency of the upper lid muscles to relax, an explanation which has since been rejected by Thurol (44).

† The amount of ptosis is clearly not a direct measure of the tone in one of two muscles which act in parallel to raise the lid. Thus an absence of ptosis might result from a lack of sympathetic tonus on the normal side, or from a maximal retraction of the lids by the levator muscles unaided by the tarsals; and a constant tonus of the smooth muscle would presumably cause a greater retraction when the lid was long than when it was short.

I am indebted to Theodore Hamblin, Ltd., for constructing this instrument.

The instrument (Fig. 1) is designed, as in Hertel's model, to allow the observer to stand in front of the patient and, viewing the lateral aspect of each eye by means of inclined mirrors, to compare the position of each with the graduations of a scale. Thus, the instrument carries a central frontal contact A and, on either side, an inferior orbital contact B, a mirror C, inclined inward for viewing the eye, and a second mirror D, above the first but inclined outward for viewing the scale E, which is mounted more laterally. Also, mounted above and at different distances in front of each orbital contact is a pair of cross-wires, as foresight F, and backsight G, to align on the eye. In addition, the whole of one side of the instrument can be slid laterally away from the other.

In making a reading, three stages are necessary. Firstly, the instrument must be set for the particular patient. The two ends are first slid apart until both pairs of cross-wires can be aligned with the centres of the patient's pupils, the eyes being directed forward. This inter-pupillary distance is then read from a scale H. The frontal contact, its bracket I adjusted to mid-way between the pupils by a scale J, is so fixed by an antero-posterior movement measured by the scale K, that exophthalmos is measured in a forward direction which shall, moreover, be constant. For this setting of the instrument, once obtained, is recorded and used for all readings on the same patient.

Secondly, it is necessary to ensure that the position of the contacts on the face shall be reproducible in subsequent readings. The position used is such that both pairs of cross-wires are aligned at once on the centres of the pupils while the eyes are directed forward. The patient is instructed to look at that eye of the observer which is open, the latter then opening first one and then the other eye in aligning the two pairs of cross-wires. In this way, when the observer's left eye, a pair of cross wires and the patient's right eye are in line, each eye is viewing the other and the axis of the patient's eye is in a constant direction relative to the instrument.* The contacts are held against the face in this position using equal pressure.

Thirdly, the actual reading is taken, using the same device to ensure that the line of vision of the eye observed passes through both cross-wires. The observer's eye, now, however, glances somewhat laterally to observe the side view of the eye in the inclined mirror C, and compares the position of the apex of the cornea with millimetre graduations of the scale E visible in the other mirror D above. The horizontal wires of all the cross-wires are coplanar with the upper edges of the mirrors in which the eyes are seen, and with the lower edges of those for the scales. Therefore, when the cross-wires are aligned on the pupils, the surface of the cornea is cut at its anterior pole by the edge of the mirror, its vertical surface at this point being readily comparable with the scalar divisions. The vertical wires on each side are as far horizontally from the intersection of the mirrors as is the scale E. Thereby errors of parallax in reading are eliminated, since the centre of the pupil and hence the apex of the cornea are in line with these wires.

A necessary precaution in taking a reading arises because the position of the instrument on the face is dependent upon that of the pupils. It is important, therefore, that the patient should not be looking laterally, and that the eyes should have a constant elevation. A convenient method is that as the patient looks at the observer, the latter should see the tips of the ears in line with the orbito-palpebral sulci. Errors in this position will be detectable by an apparently false setting of the frontal contact.

This instrument has advantages over those using the lateral orbital margins, particularly in comfort for daily readings in Graves' disease, and for ease of reading on very proptosed eyes. It carries, however, brackets in which lateral orbital contacts can be fitted. Repeated readings on each eye of two normal males over 15 days gave errors about the averages as shown:—

Difference of individual measurements from final average in mm.	—1	— $\frac{1}{2}$	— $\frac{1}{4}$	— $\frac{1}{4}$	0	+ $\frac{1}{4}$	+ $\frac{1}{2}$	+ $\frac{1}{2}$	+1
Frequency of this difference in 162 measurements...	1	0	13	45	54	34	10	5	0

SUMMARY AND CONCLUSIONS.

1. Stimulation of the cut cervical sympathetic may cause exophthalmos in certain animals. It does not do so in man, and enophthalmos does not result from section of the nerve.

2. Stimulation of the sympathetic causes retraction, and its section causes relaxation of the upper and lower lids in man.

3. An instrument is described for measuring changes in exophthalmos.

* Small metal shields L prevent the patient from viewing with his other eye.

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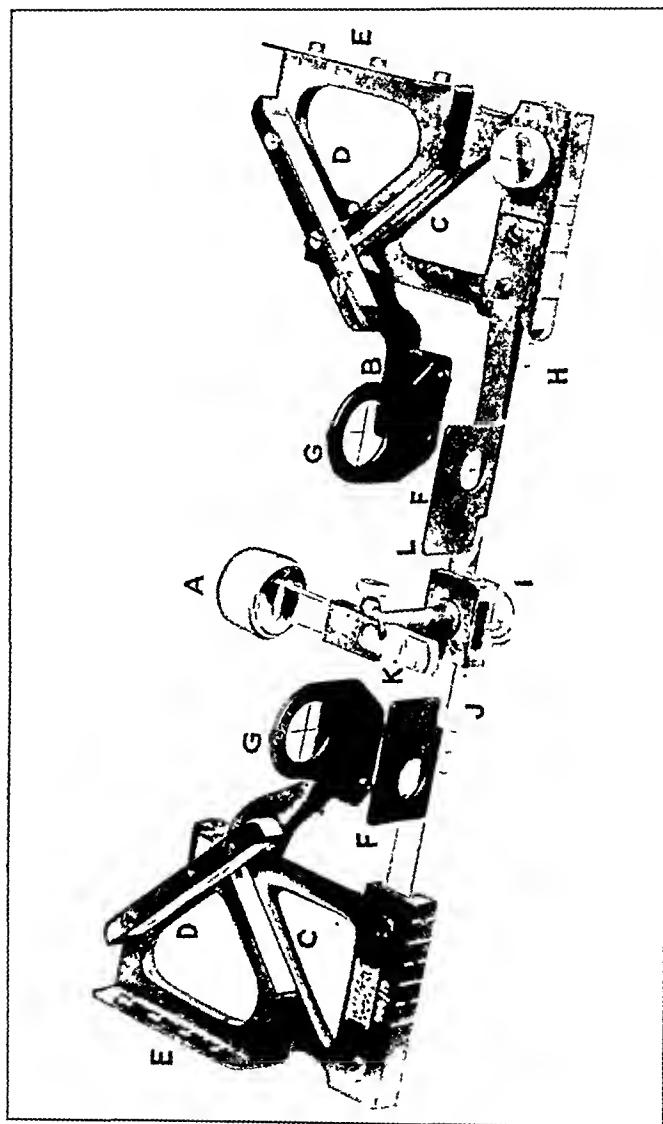


Fig. 1. The exophthalmometer, 7/12 natural size.

THE MECHANISM OF LID RETRACTION IN GRAVES' DISEASE.*

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RETRACTION of the upper lid may arise in a number of different ways, and it is important to determine which mechanism is responsible in Graves' disease. Two possible causes may be excluded immediately. Firstly cicatrisation or mal-development of structures in the upper lid may cause lid retraction, particularly on looking downward (1), but in the cases of Graves' disease such lesions are not found, and would be hard to reconcile with rapid variations in the retraction, or with its persistence while the subject is looking upward.† Secondly, weakness of the orbicularis oculi muscle has been regarded as at least a contributory cause of the retraction.‡ There is, however, no clinical evidence of orbicularis oculi weakness, while in facial palsy the disposition of the lids differs from that seen in lid retraction in Graves' disease. In 15 cases of unilateral facial palsy, I have found the exact position of the lids to vary, apparently under gravity, with the position of the head; but when the face is vertical, the lower lid lies about $1\frac{1}{2}$ mm. below that on the normal side, while the upper lid is normal in position or only slightly raised. When the face is horizontal both lids are displaced backward from their normal position. It is evident that lid retraction is not due primarily to weakness of the orbicularis oculi, and there seems to be no clear evidence to suggest such weakness as a contributory cause. The retraction of Graves' disease is therefore to be ascribed to the only remaining cause, an increased tone of one of the two muscles which raise the upper lid, namely, the striated levator palpebræ superioris muscle or the smooth superior tarsal muscle. It is the purpose of this paper to contrast retractions of these two origins, and to compare with them that arising in Graves' disease.

* Work undertaken on behalf of the Medical Research Council.

† It is not true, however, as Goldham (15) and St. Martin (34) have claimed, that wrinkling of the skin of the lid on looking downward indicates an active muscle spasm, as this wrinkle may be seen in cases having fibrous cicatrices (1) or after operation for ptosis (32).

‡ Vigouroux (44) found the facial muscles in Graves' disease difficult to excite electrically, and Poos (31) confirmed the observation for the affected side in a case of unilateral retraction. Poos (31) has assumed that the smooth and striated levators of the lid are in spasm and that the orbicularis oculi is reciprocally inhibited. I have been unable to find work on reciprocal innervation between these muscles, except that Sherrington (37) found no evidence of it in the bonnet monkey.

The levator palpebræ superioris is a striated muscle, innervated by the oculomotor cranial nerve. It arises from the lesser wing of the sphenoid above the optic foramen and passes forward behind, and then above the eye; it is inserted by multiple tendons into the lowest 8 mm. of the skin of the upper lid, into the free border of the lid, into the lowest part of the anterior surface of the tarsal plate and laterally (47). (See Fig. 1).

The superior tarsal muscle, although described by H. Müller (27), is not to be confused with the retro-ocular orbitalis muscle that he described

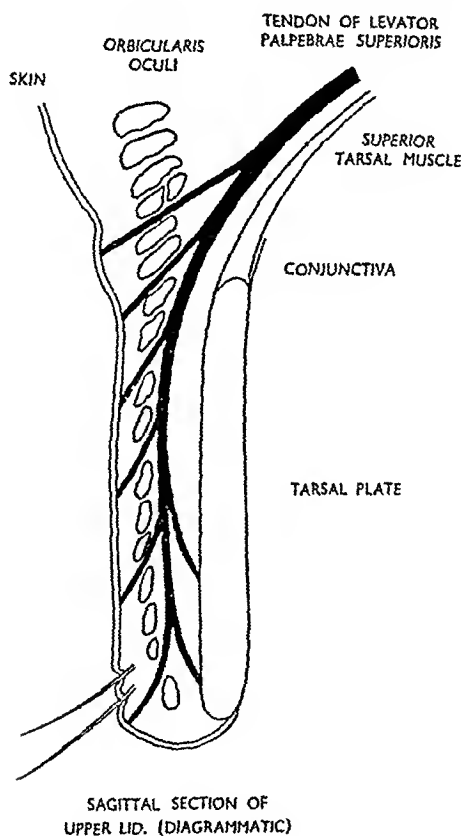


Fig. 1.

in the previous year (26), which is vestigial in man but was at one time thought to be capable of causing human exophthalmos. The superior tarsal muscle is a smooth muscle innervated from the cervical sympathetic. It passes forward from among the fibres of the levator palpebræ and in the midline of the lid inserts itself only into the upper border of the tarsal plate, which in turn is attached to the skin of the lid at its free lower border, the anterior surface of the tarsus being unattached to the superjacent skin (18). A similar inferior tarsal muscle arises from near the insertion of the inferior rectus and is inserted into the tarsus of the lower lid.

The superior tarsal muscle therefore raises the lid by traction on its free lower border through the tarsus, while the levator palpebræ is inserted also into the skin superjacent to the tarsus.

Phenomena associated with lid retraction of sympathetic origin.

This subject has been discussed in detail in a separate paper (30). When the cervical sympathetic is stimulated electrically, retraction of the upper lid is associated with a retraction downward of the lower lid relative to the cornea. The position of the globe is unchanged.

Phenomena associated with lid retraction of striated muscle origin.

Cases of levator palpebræ spasm due to cerebral lesions are rare, and are usually bilateral. I propose to illustrate the effects of lid retraction of striated muscle origin by the phenomena in voluntary staring, and by two cases in which lid retraction was regarded as due to spasm of the levator palpebræ superioris.

Staring. In cases of Horner's syndrome due to division of one cervical sympathetic chain at operation, the act of staring apparently remains unimpaired, the normal and ptosed lids being about equally raised, as measured from photographs. Thus, it is clear that staring is primarily a function of the striated levator, and I have found no evidence that the smooth muscle makes any contribution (30).

The movement of the globe in staring has been accurately studied by a number of workers, whose results are collected by Birch-Hirschfeld (6); the action is accompanied by a forward and a small downward displacement of the eye. The forward movement is of about 1 mm. as is readily confirmed by ordinary exophthalmometry. The downward displacement, which may be associated subjectively with a small shift in the visual image, results in an elevation of the lower lid relative to the cornea. The actual movement of the lower lid appears to be mainly lateral, although it may also be raised absolutely. When staring is performed while the eyes are directed downwards, the oculo-palpebral sulcus may be seen to deepen.

Case of Gunn's phenomenon. Gunn (17 and see 38) described a case in which voluntary movements of the jaw to one side were accompanied by retraction of the upper lid on the same side. The cause of the phenomenon is unknown, but is ascribed to an abnormal innervation of the somatic levator of the lid from the centres subserving movements of the jaw. I have examined one such patient whose right upper lid retracted briskly whenever he moved the jaw to the right or downwards. He had no ptosis or other neurological defect. When the lower lid was observed during the procedure, it was seen to move slightly and sharply upward relative to the cornea as the upper lid moved up, and to return when it returned. No observation was made as to whether the lower lid was actually raised or the globe depressed. When the subject was asked to make the appropriate jaw movement while

an exophthalmometer was held in position, the eye on the affected side moved forward through $\frac{1}{4}$ to $\frac{1}{2}$ mm., the amount of the upper lid retraction being about 2 mm..

Case of myasthenia gravis. Cases of myasthenia gravis occasionally present retraction of the upper lid (11) apart from that which may immediately follow prostigmine therapy. The case examined was one with left ptosis, right upper lid retraction revealing sclera above the cornea and defective movement of both eyes, particularly of the left eye upward and outward. The retraction persisted when the left eye was obscured. The right lower lid lay at a higher level than the left when the eyes were directed forward, in which position and using coloured glasses, there was no diplopia. When the eyes were closed a fold was evident on the skin of the right upper lid but not on the left. Prostigmine was given, and after 12 minutes ocular movements were normal except for slight limitation of upward movement of the left eye. The ptosis of the left upper lid and the retraction of the right had diminished *pari passu* and disappeared, and the lower lids were then equal in level. At the same time, the skin of the closed right upper lid was completely unwrinkled.

Thus in staring, and in these two cases in which the retraction may reasonably be ascribed to spasm of the striated muscle, the lower lid is raised relative to the cornea, in contrast with cases of smooth muscle spasm in which it is lowered.*

Phenomena associated with lid retraction in Graves' disease.

In a previous paper (29) I described a series of cases presenting unilateral retraction of the upper lid, and the conclusions, which have been confirmed by further cases, may be summarised. A comparison of the two eyes showed that, on the side on which the upper lid was retracted, the lower lid was raised relative to the cornea. Thus when the eyes were so directed that the lower lid margin lay at the limbus on this side, a strip of sclera was visible below the cornea on the normal side. Diplopia has never been present in this position. I have now examined 12 cases of unilateral lid retraction unassociated with proptosis in Graves' disease, and in 9 there has been a clear elevation of the lower lid on the affected side. In 3 cases there were differences of less than $\frac{1}{2}$ mm. between the lower lids, such as may occur between normal eyes. Table I summarises the cases, and 5 others in which the affected eye was also proptosed. In the latter group the affected lower lid has twice been the lower in position, which may be attributable to the proptosis, which in itself certainly produces such an effect (29). The mechanism of the elevation of the lower lid relative to the cornea was quite

* A similar disposition of the lids was seen in a case of unilateral upper lid retraction dating from birth and is shown in published photographs of such cases (10, 13, 40). At the age of 52 (Fig. 2 and 3), and in a photograph at the age of 12, the lower lid on the affected side was seen to be raised, by about $\frac{1}{4}$ mm. relative to the cornea. This was due to downward displacement of the globe.

TABLE I.*

Case.	Age.	Sex.	Graves' disease.	Proptosis of affected eye.	Elevation of : upper, lower lid.	
				mm.	mm.	mm.
1	25	F	Yes	+ $\frac{1}{2}$	1 $\frac{1}{2}$	+ $\frac{3}{4}$
2	42	F	Subsequently	+1	2	+1
3	56	F	Yes	+1	1 $\frac{1}{2}$	+ $\frac{1}{2}$
4	52	F	Previously	+1	3	+ $\frac{3}{4}$
5	42	F	Previously	0	1 $\frac{1}{2}$	+ $\frac{1}{2}$
6	36	M	Yes	+ $\frac{1}{2}$	1 $\frac{1}{2}$	+1
7	29	F	Probable early	+ $\frac{1}{2}$	2 $\frac{1}{2}$	0
8	20	F	Probable	-1	2	+1
9	30	F	Yes	+1 $\frac{1}{2}$	5	+1
10	34	F	Yes	-1	1 $\frac{1}{2}$	+ $\frac{3}{4}$
11	39	F	Yes	-1	1 $\frac{3}{4}$	+ $\frac{3}{4}$
12	18	F	Yes	+ $\frac{1}{2}$	1	+ $\frac{1}{2}$
13	48	F	Probable	+2 $\frac{1}{2}$	3	+1
14	32	F	Previously	+2	2	+ $\frac{3}{4}$
15	48	M	Previously	+2	2 $\frac{1}{2}$	+ $\frac{1}{2}$
16	36	F	Probable	+2 $\frac{1}{2}$	2	+ $\frac{1}{2}$
17	35	F	Yes	+4	2	+ $\frac{3}{4}$

* The measurements of position of the lids have been made from photographs, *camera lucida* projection, or with parallel bladed calipers.

obvious in several of the cases of the first group, in which the globe of the eye clearly lay at a slightly lower level on the affected side. This phenomenon was first reported by Hill Griffith (16, and see 19, 23) in 1886 in a case of unilateral retraction. It may readily be confirmed that a mechanical depression of the normal globe leaves the absolute position of the lower lid unchanged, and it seems likely that this slight downward displacement of the globe accounts for the relative elevation of the lower lid. It is also evident in many cases that the external canthus of the eye is raised, as occurs in voluntary widening of the palpebral fissure and an actual elevation of the lower lid might result from this cause, or possibly from slight unilateral orbicularis oculi spasm due to exposure of the supra-corneal sclera.

The retracted upper lid appeared to be hitched up at a certain level, for the skin below this level was tense and smooth, while that above was slack and over-hung in a small fold. This was best seen when the downcast eyes were rotated slowly upward, when the fold formed sooner on the affected than on the normal side; but the difference was often evident when the eyes were closed. It can be shown that the level of folding corresponds to the upper limit of the insertions into the skin of the levator palpebræ superieris, found by dissections in 15 eyes to lie from 5 to 11 mm. above the free border of the lid, with an average of 8 mm. and large variations even between the two eyes of the same individual. In the cases of retraction in Graves' disease this level between the tense tarsal skin below and the slack and folded skin above, varied from 4 to 11 mm. with an average height of 7 mm., and similar values were obtained in cases of bilateral retraction. Further, when such a patient with unilateral retraction begins to open the eyes, it is the existing fold on the retracted lid which deepens. Such a fold is present in a similar position on most normal lids when the eyes are moderately open, and in some cases persists when the eyes are looking down or closed. The same unilateral wrinkling was observed in the case of myasthenia described above. Conversely, in cases of complete ptosis from unilateral oculomotor palsy, it may be found when the eyes are closed that the sulcus and the tenseness of the tarsal skin are abolished, although present on the normal side (see Fig. 4). It is not permissible, however to accept this indrawing of the levator palpebræ insertion in Graves' disease as definite evidence that the spasm is of this muscle. When the smooth muscle is excited by cocaine, a similar wrinkle

appears slightly sooner on the retracted lid when the downcast eyes are rotated slowly upward; and a little later than normal on the ptosed lid in Horner's syndrome.* There is indeed a contrast between the slight differences of these cases and the clear folds with stretched tarsal skin below in Graves' disease (29) which suggests that in the latter condition the retraction is effected by the levator palpebræ of which the insertion is thus retracted, but quite evidently no conclusive argument can be based upon the presence or situation of the wrinkle.

Discussion.

When retraction of the upper lid is due to sympathetic stimulation, the lower lid is displaced downward on the affected side; but when it is due to a striated muscle spasm the lower lid is displaced upward, relative to the cornea. It has been shown that in Graves' disease this relative displacement of the lower lid is upward, and therefore it is to be concluded that the phenomenon is here due to a striated muscle spasm and not to over-activity of the sympathetic. In rejecting the latter explanation, which has been widely held, such evidence as has been adduced in its support may be dealt with briefly.

The sympathetic theory of lid retraction in Graves' disease has been advanced on grounds of general probability, on the incorrect assumption that sympathetic stimulation leads to exophthalmos in man as it does in animals, and on the observation that the lower lid may be depressed in Graves' disease.† I have seen such depression of the lower lid only in the presence of exophthalmos of which it is a mechanical consequence. It has further been urged that sympathectomy abolishes or relieves the exophthalmos of Graves' disease, but there are no measurements to substantiate this and published photographs (4, 9, 33) make it evident that relief of lid retraction has been confused with it.‡ Such narrowing of the palpebral fissure will of course result whether sympathetic tone was previously high or not, being a normal consequence of sympathectomy.

The present observations could only be reconciled with a theory of sympathetic overactivity by assuming that in the cases described, which were chosen as having lid retraction on one side only and no proptosis, the upper and not the lower lid muscle had become involved by sympathetic overactivity, owing to a difference between them in threshold to stimulation. It has always been a difficulty of a sympathetic theory that the pupil is not dilated, as judged from cases of Graves' disease with either unilateral or bilateral (25) lid retraction, and a similar assumption is made of differing

* Presumably the smooth muscle, although it draws up the margin of the lid, by taking load off the striated muscle may allow the latter to shorten passively under its own elasticity, and so draw in its site of insertion (see Fig. 1).

† Most previous attempts to distinguish upper lid retraction of smooth and striated muscle origins have been based on the assumption that these phenomena in Graves' disease illustrated the smooth muscle type, to be contrasted with a striate muscle type without retraction of the lower lid, usually (21) without exophthalmos and sometimes associated with ophthalmoplegia (20, 22, 24, 35, 41, 45, 46).

‡ Shaw who makes the distinction states that sympathectomy relieves retraction rather than exophthalmos (36).

thresholds for pupil and lid to sympathetic stimulation.* But the position becomes even more difficult when the lower lid is found to be unaffected. In fact, when it is stated that sympathetic stimulation does not cause exophthalmos in man, it is clear that there is only a superficial resemblance between the exophthalmos and upper lid retraction of Graves' disease, and the mydriasis and retraction of both lids produced in man by sympathetic stimulation.

The striated muscle theory of lid retraction. Spasm of the levator palpebræ may result from lesions in various situations in the nervous system and, although no such lesions are found in Graves' disease, a striated muscle origin for the retraction has often been urged. It has been emphasised that three signs occurring in Graves' disease, upper lid retraction, defective convergence and infrequent blinking, may all arise from a focal lesion in the region of the anterior corpora quadrigemina and the posterior commissure, with resulting overaction of the levator palpebræ; a similar diencephalic origin has been suggested to explain this association of signs in Graves' disease (12, 28, 42, 43).

It is not clear whether infrequent blinking in Graves' disease is due to blinks being initiated rarely, or rarely completed, for retraction itself may limit the amplitude of blinking. By examining under the microscope the negative of a slow-motion film, I have found blinks in a case of unilateral lid retraction in Graves' disease to be considerably reduced in amplitude though not in frequency on the affected side.

It has also been stressed that the function of other striated ocular muscles may be disturbed in the ophthalmoplegias of Graves' disease, and lid retraction is commonly associated with exophthalmic ophthalmoplegia (7) in which lesions are found in the muscles. The behaviour of the lids in some cases of Graves' disease is similar to that seen in patients with defective upward movement of the eyes. In the latter group, the upper lids may become fully and abnormally retracted as the eyes reach their limit of upward rotation, and this retraction passes off when the gaze is directed below the horizontal. The phenomenon is ascribed to over-innervation of a weak superior rectus muscle and simultaneously of the levator palpebræ which has a physiologically associated action (3, 28). In the cases of Graves' disease as the eyes are rotated upward, the upper lid maintains an approximately normal relation to the cornea until the eye is looking forward, when the upper lid moves rapidly upward and becomes markedly retracted (14). The order of events may be reversed on the downward route at the same or a rather lower level, the retraction passing off while the eye rotates through a small angle. This may occur in cases without limitation of upward ocular movement.

There is, however, no clear evidence localising a causative lesion in Graves' disease, and it may be of value to name the possible sites of lesions

* There is no evidence for such a difference in thresholds either clinically in man or to electrical stimulation in animals (30). Justin Besançon (5) postulates an added para-sympathetic stimulation, and has shown that in dogs simultaneous injection of pilocarpine and ephedrine produces lid retraction without mydriasis.

producing lid retraction in other conditions. When due to a focal lesion in the central nervous system, the retraction is usually associated with a limitation of ocular movement in the vertical directions (22). This limitation may be of two types, involving movement in response either to all stimuli or only to voluntary effort, various reflex movements being preserved. In the former and more common type, lesions are usually situated in the region of the posterior commissure above, but not involving the oculomotor nuclei. In the latter type (2, 8, 28, 35, 39) bilateral lesions are probably present in the cortex or in the internal capsules. In both cases the retraction is ascribed to interruption of tracts running from the cerebral cortex to the oculomotor nuclei. Two observations suggest that the retraction in Graves' disease differs in origin from either of these types. Firstly, I have examined three cases of Graves' disease in which lid retraction was associated with grossly defective voluntary upward movement of the eyes, which had a range of rotation of only about 10° above Reid's base line, as compared with a normal range of 30° , but, in none of these cases was the range of associated ocular movement greater than the voluntary range, either in following a moving finger, or on flexion of the head while the gaze was fixed on a stationary object or accompanying attempted closure of the eyes against resistance. Secondly I have examined the posterior and habenular commissures of two cases which died of Graves' disease having had bilateral lid retraction, but have found no evidence of degeneration. The patients were females of 26 and 61, one of whom died at operation and the other in heart failure. One had exophthalmos and lid retraction, the other lid retraction alone. In both cases the pineal gland was normal in size and attached to the habenular commissure. In one a large saddle of calcification on the anterior aspect of this commissure produced no apparent constriction or deformity, while in the other a small and normal area of calcification was present in the same situation. Kulchitsky-Pal staining of cross-sections of the posterior and habenular commissures showed no evidence of demyelination in either commissure as compared with controls, the frequency and diameter distribution of fibres being sensibly normal.

A number of unlocalised lesions, for example in tabes dorsalis or encephalitis lethargica may cause retraction of the lid, but in these cases the muscle responsible for the retraction has not been established. Retraction may follow regeneration of a damaged oculomotor nerve. It may also be caused by spasm of the levator palpebræ arising either directly in tetany or myotonia congenita; or in association with weakness of the superior rectus on the same side, both these co-ordinated muscles presumably being over-innervated as a result of the weakness of one. There is no evidence in Graves' disease to indicate any such cause or site of origin for the spasm of the levator palpebræ, but investigation on these lines should be of more value than the assumption of sympathetic over-activity, which appears to explain neither exophthalmos nor lid retraction as it occurs in this condition.

SUMMARY.

Retraction of the upper lid in Graves' disease differs from that produced by sympathetic stimulation, but resembles that caused by spasm of the striated muscle, levator palpebræ superioris. Thus the lower lid is lowered on sympathetic stimulation, but raised relative to the cornea when the upper lid is retracted by levator palpebræ spasm or in Graves' disease. Sympathetic over-activity is therefore rejected as a cause for the lid retraction.

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Fig. 2. Left congenital lid retraction.



Fig. 3. Left congenital lid retraction.



Fig. 4. Left third nerve palsy.



OBSERVATIONS ON THE OXYGEN CONTENT OF VENOUS BLOOD FROM THE ARM VEIN AND ON THE OXYGEN CONSUMPTION OF RESTING HUMAN MUSCLE.*

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THE following paper records an attempt to estimate the oxygen consumption of resting human muscle by correlating the blood flow through the forearm with the oxygen content of blood obtained from the median vein in the antecubital space.

Method.

The forearm blood flow was measured plethysmographically in the way recently described by Grant (5) which, as he showed, measures chiefly the blood flow through the forearm muscles. Blood was withdrawn from the median vein in the antecubital space just above its junction with the deep median vein which arises from the muscles. Observations were confined to subjects in whom the median vein obviously received a large branch from the deep tissues. It was found that there is no difference between the oxygen content of blood taken whilst the blood flow is being recorded and that of blood taken between two successive records; the duration of the venous obstruction during the recording of the inflow of blood being only about 12 seconds and therefore too short to cause any significant degree of congestion. In practice, three measurements of blood flow were made at intervals of 20 seconds immediately before and after taking a blood sample. The mean of the six blood flows was taken as indicating the rate of flow corresponding to the blood sample.

A word is required about the precautions observed in withdrawing and storing the blood samples. To avoid sensory stimulation which may cause vasodilatation in the forearm (Grant (5)) the needle was inserted through skin locally anæsthetised with 0.1 c.c. 2% procaine. The needle was left in the vein, clotting and loss of blood being prevented by attaching the needle by an adaptor and rubber tubing to a reservoir of 3 per cent. sodium citrate. The citrate was allowed to enter the vein at a rate of about 2 c.c. in 30 minutes,

* Work undertaken on behalf of the Medical Research Council.

being controlled through a Murphy drip. The rate of inflow is insufficient materially to dilute the blood in the vein but as a precaution against withdrawing diluted blood, about 1 c.c. of blood was allowed to flow from the needle after removing the adaptor and before collecting a sample.

The blood samples (of about 5 c.c.) were taken into closely fitting glass syringes of which needle and dead space were filled with aqueous heparin solution. Since Looney and Childs (10) have pointed out that a covering layer of paraffin oil is unsatisfactory for preventing absorption of oxygen by blood from the air, the samples were transferred immediately to glass tubes containing mercury until analysed. The tubes used were similar to that described by Peters and Van Slyke (16). At the lower end they had a two-way stopcock so that they could be detached from the mercury reservoir and on re-attachment the air trapped below the lower stopcock released through the side tube. Blood kept in such a way shows no alteration in oxygen content at the end of four hours.

The oxygen content and the total oxygen combining power of the samples of blood were determined by the method of Van Slyke and Neill (18) within four hours of collection.

All determinations were done in duplicate on 1 c.c. samples and if these results differed by more than 0.5 volumes of oxygen per cent., in triplicate.

Knowing the blood flow and the difference of oxygen content of the arterial and venous blood it is clear that the oxygen consumption of the forearm can be estimated. For this purpose arterial blood was assumed to be 95 per cent. saturated with oxygen. The method of calculation is illustrated by the following example:—

R.E.S. 27.6.38.

Average blood flow = 1.23 c.c. blood per 100 c.c. limb volume per minute.

O₂ content of fully saturated blood ... = 21.1 c.c. per 100 c.c..

O₂ content of arterial blood (95% Sat.) ... = 20 c.c. per 100 c.c..

O₂ content of venous blood = 10.75 c.c. per 100 c.c..

Therefore each 100 c.c. of arterial blood loses

20 - 10.75 c.c. O₂ = 9.25 c.c. O₂.

or 1 c.c. of blood loses 0.0925 c.c. O₂.

Blood flow = $\frac{1.23 \times 60}{100}$ c.c. blood per c.c. limb vol. per hour.

Oxygen usage = $\frac{1.23 \times 60 \times 0.0925}{100}$ c.c. O₂ per c.c. limb vol. per hour.

= 0.69 c.c. O₂ per 1 c.c. (or per g.) per hour.

To render the values comparable with those obtained by other workers from animal experiment the oxygen consumption of the forearm is expressed

as c.c. oxygen removed by 1 g. of forearm tissues per hour; the specific gravity of the forearm tissues being approximately that of water, one cubic centimetre may be taken as equivalent to one gramme.

Since the forearm blood flow is mainly muscle blood flow and the blood in the median vein when the hand circulation is arrested comes mainly from the muscles, the values obtained for the oxygen consumption of the forearm must nearly represent that of the forearm muscles. That the median vein receives but a small contribution from the forearm skin can be shown as follows. In the first place, the circulation to the hand being free and the hand warm, if the forearm veins are collapsed by passively elevating the limb then on lowering the arm to a dependant position all the veins fill in about 15 seconds. But, if the circulation to the hand is arrested to exclude the large venous return from the hand and the elevation and lowering of the limb repeated, then the median vein above its deep branch again fills in about 15 seconds whereas the median vein below the branch and the other cutaneous veins of the forearm remain collapsed for a minute or longer. Secondly it is found that a large increase of the oxygen content of the blood in flowing through the forearm skin causes but little change in the oxygen content of the median vein blood. For example, in one subject at rest, the circulation to the hand being arrested, the oxygen saturation of blood withdrawn from a cutaneous vein of the forearm was 40%. The cutaneous blood flow was then greatly increased by pricking 10% morphine solution into numerous spots on the forearm; when the flare was at its height a second sample of blood was withdrawn from the same vein and was found to have an oxygen saturation of 93%. The oxygen saturation of blood from the median vein, which was 55% before the flare, was raised only to 65%. It is clear therefore that blood flowing through the forearm skin influences but slightly the oxygen content of the median vein blood.

There is at the moment no direct way of estimating the oxygen consumption of the forearm skin and bone, but skin and bone exist in approximately the same relative proportions to each other in the fingers (6) and since for them an expression of upper limit of oxygen consumption can be obtained, it is possible to obtain from this the probable order of the oxygen usage of the forearm skin and bone. Thus Goldschmidt and Light (4) found no difference between the oxygen content of arterial blood and of venous blood from the fingers in a state of maximum vasodilatation. Since an arteriovenous oxygen difference of 0.5 c.c. of oxygen per 100 c.c. of blood should be recognisable, this figure may be taken as the upper limit of arteriovenous oxygen difference under these conditions, when blood flow to the fingers is 30 to 40 c.c. per 100 c.c. finger volume per minute (Grant and Pearson (6)). From these figures the oxygen usage of the fingers would be 0.09 c.c. oxygen per c.c. of finger per hour. If this result is applied to the above example in which the oxygen consumption was .069 c.c. oxygen per g. of forearm per hour, and we consider that 15% of the forearm consists of skin and bone (5) using oxygen at the rate of .09 c.c. per g. per hour, then the

remaining 85% of muscle was using oxygen at the rate of .065 c.c. per g. per hour. On the other hand, if the oxygen metabolism of the skin and bone of the forearm be presumed to be negligible, the figure .069 c.c. oxygen per c.c. forearm per hour should be expressed as .069 c.c. oxygen per 0.85 c.c. muscle per hour, so that the corrected muscle metabolism would be .081 c.c. oxygen g. per hour. The actual metabolism of the muscle in this instance would lie between .065 and .081 c.c. oxygen g. per hour. Until the oxygen usage of the skin and bone of the forearm is more accurately known it would be useless to correct the values obtained in the above manner. That neglect

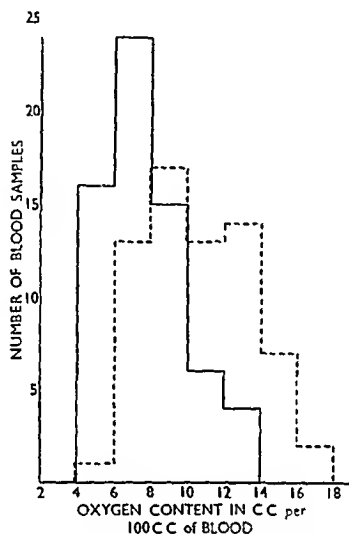


Fig. 1. Distribution curve of the oxygen content of 65 blood samples drawn from the median vein with the circulation to the hand arrested (continuous line) compared with that of 67 blood samples withdrawn from an arm vein, the circulation to the hand being free (broken line, Looney and Freeman's (11) observations).

of this factor has but little influence on the results is suggested by the fact that there is no correlation between the oxygen consumption of the forearm and the proportion of skin and bone to muscle in that forearm. We may accept then that the values obtained do nearly represent the oxygen consumption of the forearm muscle.

Oxygen content of blood from the median vein with the circulation to the hand arrested.

It is well known that blood withdrawn from an arm vein when the circulation to the hand is free varies greatly in oxygen content. Thus Keys (9) in observations on 63 normal subjects (blood stored under paraffin) found the average percentage oxygen saturation to be 68.2% with a range of 25% to 85%. I find, however, that when the circulation to the hand is

arrested, the mean oxygen content is considerably lower and the range is less. Thus in 63 observations on 16 normal subjects the mean value of oxygen percentage saturation is 41%, the highest being 74%, the lowest 22%. The data supplied by the observations of Looney and Freeman (11) provide a series more comparable with the present one since the blood, obtained from the arm veins of 67 normal patients with normal circulations, was stored over mercury. These workers find the mean value for the oxygen content of blood from the forearm to be 10.6 e.e. of oxygen per 100 e.e. (the maximum being 17.5 e.e. and the minimum 4.0 e.e.). But I find that when the circulation to the hand is arrested (65 observations on 18 subjects) the mean oxygen content is 7.9 e.e. per 100 e.e. (the maximum being 14 e.e., the minimum 3.5 e.e.). The difference between these results is shown in Fig. 1. That the present series includes as many as eight observations on

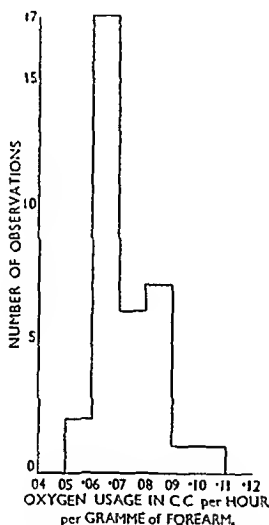


Fig. 2. Distribution curve of the oxygen usage of the forearm obtained from 34 observations on twelve normal subjects.

the same individual does not invalidate the comparison since it was found that the variation between a number of observations on the same person was comparable to that found in the series as a whole. The fact that the two series of results were obtained in different countries and not by the same observer may in part, but not entirely, account for the difference between them.

The reason for a difference becomes clear if it is remembered that the blood from the superficial veins of the forearm is derived mainly from the hands and that the hands contain large numbers of arteriovenous anastomoses which, when open, allow arterial blood to flow directly into the veins. Goldschmidt and Light (4) showed that arterial blood can be obtained from

the veins on the back of the hand if the hand is adequately warmed, while Meakins and Davies (13) found that almost arterial blood can be obtained from an arm vein under the same conditions. The effect of the venous return from the hand is shown by withdrawing samples of blood from the median vein when the circulation through the hand is free and when it is arrested by means of a cuff on the wrist. For example in one subject when the hands were cold (that is blood flow being low), the oxygen saturation of median vein blood was 37% when the circulation to the hand was free, and 36.5% when the wrist cuff was inflated to 200 mm. Hg.. When the hands were warm (the hand blood flow being now high) the saturations were 85% and 36% respectively. It is clear therefore that the circulation through the hand is an important factor influencing the oxygen content of blood obtained from a vein of the forearm, and circumstances leading to constriction of the hand vessels will result in a lower oxygen content of blood in the forearm vein, circumstances leading to dilatation in a higher oxygen content. It should be noted that, as the above example demonstrates, when the hand circulation is arrested there is no material difference in the oxygen content of the blood from the median vein whether the subject is hot or cold. As the results show, however, that even when the venous return from the hand is eliminated the median vein blood still shows considerable variations in oxygen content, not only from subject to subject but in the same subject at different times. These variations will be referred to again later but it may be remarked that they are associated rather with variations in resting muscle blood flow than with variations in oxygen consumption of the muscle.

Oxygen usage of the muscle.

Just as it has been previously shown (Grant (5)) that the blood flow through resting human muscle is much lower than that found in animal experiment, so it is now found that the oxygen consumption is also very much lower. The mean oxygen usage of the forearm based on 34 observations on twelve normal subjects is 0.07 c.c. oxygen per g. of tissues per hour, the extremes being 0.05 and 0.10. The results are compared in Table I with those obtained for resting human muscle by Jarisch and Gaisbock (8) and those obtained by other workers from animal experiment. The results of Jarisch and Gaisbock (8) were obtained by measuring the decrease in the total oxygen consumption of a subject in the basal state when the circulation to the limbs was obstructed, the decrease representing the amount of oxygen used by the limbs. The volume of the obstructed limbs was measured and the specific gravity of muscle taken as unity so that the oxygen consumption could be expressed as c.c. oxygen per g. of muscle per hour; they assumed that 72.5% of the limb volume was muscle and that the metabolism of the remainder of the tissues was negligible.

These authors give the oxygen consumption of muscle as 0.84 to 1.0 c.c. oxygen per g. per hour, but as Asmussen and Hansen (1) point out, and I can confirm, this value is due to an arithmetical error in the use of their data,

recalculation from their data gives an oxygen usage ten times less, namely 0.1 to 0.084 c.c. oxygen per g. per hour. These results agree closely with those now obtained for the human subject. The much higher oxygen consumption in animal experiment is due possibly to the anaesthetic and operative procedures employed; Chauveau and Kaufmann's (3) observations, however, were carried out on the unanaesthetised horse and the difference here may lie in insufficiently quiescent muscles. The difference may also in part be due to a difference in species, for Meyerhof and Himwich (14), using the Warburg technique found that the oxygen consumption of the rat's diaphragm was 1.1 c.c. per g. per hour whereas that of the mouse was 2.2 c.c. oxygen per g. per hour. The variations of all the results in the human subject are shown graphically in Fig. 2. The oxygen usage varies more from subject to subject than in the same subject at different times. For example, in one subject on whom eight determinations were made on different occasions, the average usage was 0.64 c.c. per g. per hour, the highest being 0.071 and the lowest 0.060 c.c. per g. per hour.

TABLE I.

Oxygen used by muscle in c.c. per g. per hour.

Observer.	Material.	Mean O ₂ usage, c.c..	Max.	Min.
Holling	Human forearm	0.07	0.10	0.05
Jarisch and Gaisbock (8)	Human limb muscle	—	0.10	0.084
Chauveau and Kaufmann (3)	Levator labialis of horse	0.34	0.49	0.17
Verzár, Langley and Naki (19)	Cat's gastrocnemius	0.27	0.52	0.14
Himwich and Castle (7)	Dog's gastrocnemius	0.36	0.40	0.22
Barcroft and Kato (2)	Dog's gastrocnemius	0.64	3.30	0.31
Millikan, G. A. (15)	Cat's soleus	0.23	0.38	—

Comment.

The oxygen usage of muscle is in general more constant than oxygen saturation of the venous blood and it is found that variations in the oxygen saturation of venous blood are associated rather with variations in blood flow than with change in oxygen consumption. It would seem therefore that the variations in the oxygen saturation of venous blood under resting conditions are not to be attributed to alterations in the metabolism of the forearm muscles. The probability that they are to be ascribed to nervous or humoral influences acting on the blood vessels receives support from the fact that they are more pronounced in excitable than in phlegmatic subjects and that they are reduced by avoiding, so far as possible, causes of emotional

stimulation. The effect of emotional stimuli in causing an increase in the blood flow to the muscle was strikingly demonstrated in one subject who under resting conditions in the laboratory consistently had a blood flow of less than 1.5 c.c. per minute per 100 c.c. of forearm, and the blood obtained from the median vein was definitely blue in colour and had an oxygen saturation of 35 per cent. But on one occasion when he was used as a subject for demonstration at a scientific meeting the blood flow was increased to 9 to 10 c.c. per minute per 100 c.c. forearm in spite of adequate physical rest, and blood obtained from the median vein was bright red. Shortly after the demonstration the blood flows fell to almost basal figures. Such long continued effects, however, are difficult to reproduce under laboratory conditions, the convenient stimuli having too transitory and uncertain an effect on the blood flow and the oxygen content of the venous blood. An attempt to reproduce the effect of humoral influences was made by injecting adrenaline. As with the sensory stimuli so also with the intravenous injection of minute doses of adrenaline (1 gamma), the vasodilator effect is too transitory to be reflected in a definite change in the oxygen content of a blood sample from the median vein. Moreover the brief vasodilatation is followed by a constriction.*

A more prolonged vasodilatation can be provoked by infusing into a vein a weak solution of adrenaline at the steady rate of 7 to 10 gamma a minute, and is accompanied by an increased oxygen saturation of the venous blood. For example in one subject the initial basal blood flow of 1.54 c.c. of blood per 100 c.c. of forearm per minute rose to 3.29 c.c. of blood per 100 c.c. of forearm per minute on infusing 7 gamma of adrenaline per minute. The oxygen saturation of the venous blood meanwhile rose from 46.5% to 73%, the blood flow and the oxygen content of the venous blood rising together so that the calculated value for the oxygen usage of the muscle remains constant. It should be noted that it is difficult to maintain a high level of forearm blood flow in this way; in the first place minor variations in the rate of infusion cause large variations in the rate of blood flow, and secondly even though the rate of infusion is kept constant the increased blood flow subsides after a period of about three minutes. This period is, however, sufficiently long to obtain a reliable series of blood flow measurements and a corresponding blood sample. The increased blood flow to the muscles resulting from the infusion of these physiological amounts of adrenaline resembles that found from time to time in less phlegmatic subjects since it is associated with no obvious increase in the oxygen metabolism of the

* It is of interest to note that the vasodilatation may be not only followed but also preceded by vasoconstriction. The preceding vasoconstriction is not obvious in all subjects but is striking in some. For example in one otherwise normal subject 1 gamma of adrenaline injected intravenously caused conspicuous pallor and unpleasant palpitation. This was associated, thirty seconds after injection with a fall in forearm blood flow from 2.4 to 1 c.c. per min. per 100 c.c. limb volume. With the disappearance of subjective symptoms, from fifty to eighty seconds after injection, the blood flow rose to a maximum of 14.5 c.c. per 100 c.c. per minute and then fell to 1.6 c.c. per 100 c.c. per minute and slowly returned to the resting level of 2.0 c.c.. These findings in man are similar to those found by Roome (17) in the isolated muscle of the dog.

muscles but differs from it in being only of short duration. This observation is interesting in view of previous work on the calorogenic action of adrenalin and further investigations of this aspect of the question are being made.

SUMMARY.

Blood from the median vein with the circulation to the hand arrested is derived mostly from the muscles of the forearm. The oxygen consumption of human muscle can be calculated, therefore, from the oxygen content and combining power of blood from the median vein and the amount of blood flowing through the forearm muscles.

The oxygen consumption of resting human muscle is much less and slightly more constant than is found in animal experiment, being 0.07 c.c. oxygen per g. of muscle per hour (maximum 0.103 c.c., minimum 0.054 c.c.).

Observations are described on the oxygen content of blood taken from the median vein both with the circulation to the hand free and arrested. The oxygen content of the venous blood from the arm is affected by the vascular state of the hand. If the circulation to the hand is arrested the venous oxygen content is lower and rather more constant than if the circulation to the hand is free.

In any individual the variations in oxygen content of venous blood obtained with the circulation to the hand arrested are associated with variations in the resting blood flow to the forearm, the oxygen consumption remaining fairly constant. These variations are presumed to be secondary to nervous influences and are less marked in phlegmatic subjects.

Adrenalin in low concentration causes an increase in the blood flow rate with a complementary decrease in the arteriovenous oxygen difference, the oxygen consumption of the muscle being apparently unchanged.

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CURE OF EXPERIMENTAL RENAL HYPERTENSION.*

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It is well known that the permanent constriction of one or both renal arterics in the dog (8), monkey (6) or rabbit (14) is associated with persistent elevation of the arterial pressure.

The observations described below present a successful attempt to cure this type of experimental arterial hypertension by establishing an accessory circulation to the ischæmic kidney.

Methods.

Forty-three healthy dogs, weighing from 12 to 18 kg., were trained to remain calm on a table for some 15 min. prior to the arterial pressure measurements. The mean blood-pressure was recorded at intervals of 2-7 days by puncturing the femoral artery in the groin with a hypodermic needle connected to a mercury manometer (9, 15). With a little training the arterial puncture may be made without any apparent discomfort to the dog.

After a sufficient number of observations on the resting blood-pressure had been made, the dogs were operated upon aseptically under ether anaesthesia. The details of each operation will be given later. A few days after complete recovery the arterial pressure determinations were repeated.

The animals were kept under good hygienic conditions, receiving a liberal supply of well-balanced food and being allowed sufficient exercise.

Results.

The immediate effects of constriction or total occlusion of the renal artery in the unanaesthetized dog were studied by one of us (Samaan (16)) who applied to the renal artery an ebonite cuff lined with a thin rubber membrane and connected to the outside of the animal by means of a fine rubber tube. Partial compression or total occlusion of the artery was produced by injecting air into the rubber tube. Constriction or total block

* Preliminary report given to the 16th International Physiological Congress, Zürich (August, 1938). Kongressbericht III, page 77. It is a pleasure here to thank Professor G. V. Anrep for his interest and criticism of the work.

of the artery for periods up to 15 min. was associated with a slight increase of the arterial pressure, not exceeding 20 mm. Hg.. When the artery was released the blood-pressure rapidly returned to its original level.

For studying the effects of persistent constriction of the renal artery one kidney was first removed and a few days subsequently the remaining kidney was exposed through a lumbar incision and its renal artery constricted permanently to any desired degree by applying a small adjustable silver clamp similar to that devised by Goldblatt and co-workers (8).

Fig. 1 represents the typical effects on the arterial pressure of chronic renal ischaemia. Curve "A" is the average of blood-pressure changes in a group of 12 dogs, while curve "B" is that of another group of 19 dogs. It



Fig. 1. Types of blood pressure responses which follow partial constriction of the renal artery in the dog. At the arrow the renal artery was partially constricted.

In this and subsequent figures, ordinates = blood pressure in mm. Hg, abscissae = time in weeks.

A. Maintained hypertension. Average changes in 12 dogs.

B. Transient hypertension. Average changes in 19 dogs.

may be noted that in group "A" the arterial pressure began to rise shortly after the constriction of the renal artery, reached its maximum within 10 days and was maintained at this high level for several weeks. Dogs of group "B," on the other hand, presented an initial temporary elevation of the arterial pressure which lasted a few days but was soon followed by a gradual fall towards the original value, being maintained at or a little above the normal pre-operative level.

The failure of the hypertension to be maintained in the dogs of group "B" was thought to be due to an insufficient constriction of the renal artery. With this possibility in mind, the artery was exposed with the object of increasing the degree of its constriction. On inspecting the kidney large collateral blood vessels connecting it to the surrounding tissues were seen.

The question immediately arose: Did the augmented collateral circulation of the ischaemic kidney prevent the persistence of the hypertension which commonly followed renal ischaemia? In answer to this question the following experiments were made.

Effect of augmenting the collateral renal circulation on hypertension. Persistent hypertension was produced by a permanent partial constriction of the renal artery of the kidney in unilaterally nephrectomized dogs. Particular care was taken not to disturb the kidney and its surroundings during the procedure of constricting the artery. In 8 animals in which the hypertension was maintained at a nearly constant level at 180 mm. Hg. or more for 4-11 weeks, a further operation was made with the aim of developing an adequate collateral circulation to the ischaemic kidney by means of renal decapsulation and by establishing an intimate contact of the kidney with the greater omentum and, in some instances, with the spleen as well. Great care was taken to avoid, as much as possible, any undue handling of the renal vessels, ureter, suprarenal gland or of the region in which the arterial clamp was previously embedded.



Fig. 2. Effect of augmenting the collateral renal circulation. At arrow A, the left renal artery was constricted, the right kidney having been removed previously. At arrow B, renal decapsulation and reno-omentopexy.

Two of the animals died during or shortly after this operation. In the remaining six dogs the arterial pressure fell to within 20 mm. Hg. of the pre-operative level within two weeks (Figs. 2 and 3).

It may be remarked in passing that we noted in the dogs after cure of the hypertension that excitement produced a greater rise of blood-pressure than in normal dogs. Thus the entrance of a stranger into the room had hardly any effect on the blood-pressure of normal dogs but commonly caused a transient increase of about 20 mm. Hg. in the arterial pressure of dogs cured of hypertension in the manner described.

Effect of interrupting the collateral renal circulation on hypertension. The experiments just described show that the hypertension disappears after the collateral renal circulation is re-inforced. It might be argued that the apparent cure of this type of experimental hypertension is not dependent on the increase of the collateral blood supply, but is due to the handling of the kidney during decapsulation, or to some other cause.

In order to exclude this possibility the effect of interrupting the renal collateral vessels was investigated. In two dogs, apparently cured from the experimental hypertension described above, the greater omentum was carefully separated from the kidney, cutting off all the existing collateral vessels between ligatures. A very thin rubber sleeve was then slipped over the denuded kidney, and kept in position with a stitch or two, in order to prevent the re-growth of collaterals.* Great care was taken to avoid the region of the arterial clamp and the kinking of the renal vessels and ureter.

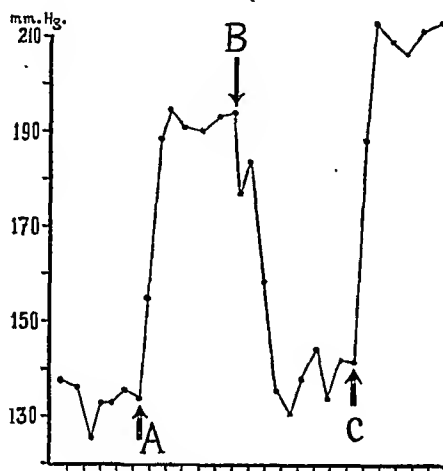


Fig. 3. Effects of altering the collateral renal circulation to the ischæmic kidney of the dog. At arrow A, constriction of the left renal artery, the right kidney having been extirpated at a previous operation. At arrow B, renal decapsulation and reno-omentopexy. At arrow C, interruption of collateral renal circulation. Details in text.

In both dogs the interruption of the omento-renal collateral vessels was followed by very marked elevation of the blood-pressure, which exceeded the values previously reached after the application of the arterial clamp (Fig. 3, A and C).

Discussion.

The view that experimental hypertension following renal ischæmia closely resembles "essential hypertension" in man is gaining a firm support (7). It is generally accepted that the earliest pathological alterations seen in "essential hypertension" involve the renal vessels. They consist either in arteriolar thickening, degeneration and arteriolo-sclerosis of the kidney (2, 5, 11) or in a narrowing of the orifice or lumen of the main renal artery (10). Such vascular changes might lead to a state of renal ischæmia comparable to that which in experimental animals provokes persistent hypertension.

* The small blood vessels running along the ureter were probably the only collaterals which were left intact.

The present investigation shows that the hypertension following renal ischæmia can be cured by re-inforcing the collateral circulation to the ischæmic organ (Fig. 2, B). Furthermore, interruption of the renal accessory vessels is followed by a pronounced elevation of the blood-pressure (Fig. 3, C). It may be of interest to mention that in our early experiments on the permanent constriction of the renal artery, the surgical technique entailed the separation of the kidney from its surroundings and its temporary exteriorization within the lumbar wound in order to facilitate the application of the silver clamp to the renal artery. Under these conditions a transient moderate hypertension of a few days' duration was the best that was obtained. When, however, the technique was modified by the application of the arterial clamp to the renal artery "in situ" without the least disturbance to the anatomical relations of the kidney, persistent hypertension was uniformly obtained.

If one accepts the view that ischæmia of the kidneys plays a part in the causation of the elevated blood-pressure of "essential hypertension," the surgical treatment described in the present paper, namely bilateral renal decapsulation and re-inforcement of the collateral circulation to the kidneys, might prove of value in early cases of essential hypertension. Chabanier et al (3) have reported that renal decapsulation and denervation is followed by definite fall of the blood-pressure in patients with chronic nephritis and hypertension. Goldblatt (7) has also suggested this operation as a possible treatment for hypertensive patients.

The operation of renal decapsulation (4, 12) and re-inforcing the collateral circulation to the kidneys does not seem difficult when compared with the extensive operations devised recently by American surgeons for the treatment of hypertension in man (1, 13).

SUMMARY.

1. Hypertension produced experimentally by constricting the renal artery in the dog can be cured by decapsulation of the ischæmic kidney and promoting the development of the collateral renal blood supply. The cure as expressed in the return of the arterial pressure to the normal level is permanent.

2. In dogs thus cured from hypertension, the interruption of the newly formed renal collateral circulation is followed by persistent elevation of the arterial pressure (Fig. 3, C).

3. The significance of the surgical cure of experimental renal hypertension and its suggested application to the treatment of "essential hypertension" in man is discussed.

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INSULIN-SENSITIVE AND INSULIN-INSENSITIVE TYPES OF DIABETES MELLITUS.*

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IN a previous series of publications it has been shown in healthy men that the efficiency with which insulin depresses the fasting blood sugar level (16) (17) (18) (19) and with which it suppresses hyperglycæmia consequent upon oral (22) or intravenous administration of glucose (17) is not fixed but is influenced by another factor, the sensitivity of the body to insulin. This demonstration disclosed the possibility that two types of diabetes mellitus might exist, one caused by lack of insulin and the other, not by lack of insulin, but by insensitivity to insulin. At first sight it would appear that this possibility could easily be put to the test by measuring the effect, in different diabetic patients, of injecting intravenously a standard dose of insulin when the patients were fasting. But this test is unsatisfactory for the action of a standard dose of insulin being proportional to the height of the blood sugar level (15) (17) (37), and the fasting blood sugar levels of different diabetic patients varying widely, the insulin depression curves so obtained would not be comparable in different patients save in the rare instances when the fasting blood sugar levels chanced to be alike. Another test was therefore sought and this was found in the insulin-glucose test (21). In this test insulin is injected and glucose given orally as nearly simultaneously as possible, and the effect of insulin in suppressing alimentary hyperglycæmia is measured by comparing the insulin-glucose blood sugar curve with the curve obtained after administration of glucose alone. It has been shown in animals (17) and in normal men (22) that the degree of the suppression effected by the constant dose of insulin is proportional to the degree of insulin sensitivity measured by an insulin depression curve. Application of this test to diabetic patients showed two types of response. In one the suppression of hyperglycæmia was normal in degree, in the other it was greatly diminished. Diabetics showing the former type of response were classified as insulin-sensitive diabetics, those showing the latter type as insulin-insensitive diabetics (21). The existence of the two types of response

* Some of the early experiments made in this investigation were done with help of Dr. Gerald Flaum of the Bellevue Hospital, New York, and Dr. D. B. McNair Scott. The expenses of part of this work were defrayed by a grant from the Lindley Diabetic Research Fund.

to the insulin-glucose test has been confirmed by Marble (32) (33) and by de Wesselow and Griffiths (7).

In this paper the results of the insulin-glucose test in diabetic subjects is reported, and the significance of the two types of response is discussed.

Methods.

Patients. Thirty-four cases of diabetes mellitus were investigated. In this group 19 were females and 15 were males, and the ages ranged from 19 to 71 years. These thirty-four diabetic patients were not a selected series and all investigations which were technically satisfactory have been included. The only considerations which have influenced the choice of patients have been first the absence of infections or gross complications, such as myxœdema, and second the patient's willingness to remain in hospital for the length of time required for the investigation.

Diet and insulin. All the patients were living in the hospital wards throughout the period of the investigation and received diets of known composition. These were cooked and weighed in the Diet Kitchen of the hospital,* and their consumption by the patient was checked by the ward sister. In the series of experiments in which the composition of the diet was varied the caloric value of the diet remained constant for all diets taken by a particular patient.

The majority of the patients required insulin, and to ensure that control with insulin was evenly maintained ordinary insulin was given in three equal or approximately equal doses each half an hour before the three main meals of the day. Protamine insulin was not used. In every case before tests were performed the daily dose of insulin necessary to control the particular case of diabetes was determined and this dose remained unaltered throughout the whole period of the investigation.

In order to ensure that the disease in the different patients was in a comparable state of control when the experiments were performed all patients were controlled until the degree of glycosuria conformed to a common standard. Urine specimens were obtained half an hour before and two hours after the three main meals, and the standard aimed at was that glycosuria should be absent from most specimens and only present in any quantity in the after breakfast specimen. Some cases became sugar free on diet alone; the majority did not, and in these insulin was given in doses sufficient to reduce the glycosuria to the desired amount. In cases requiring insulin complete freedom from glycosuria was avoided, rather it was aimed to give the minimum amount of insulin to control the disease. In this way it was hoped to avoid erroneous results from overdosage with insulin. *No tests were performed upon any patient until the insulin requirements had been constant and glucose excretion reasonably so for at least one week.* In those experiments in which the composition of the diet was changed at least one

* We are indebted to Miss E. Washington for control of these diets.

week was allowed to elapse before the effect of the new diet on the test curves was estimated.

Tests. It must be emphasized that all tests were performed under conditions as strict as those required for the determination of the basal metabolic rate. The tests were made in the fasting state at approximately 10 a.m., the patient having had neither food nor insulin since 6.30 p.m. the previous evening. The patient retired to bed at 8 p.m. and remained in bed until next morning when he was wheeled in a comfortable chair to the laboratory next the ward. Further details of the conditions which it is necessary to observe if accurate results are to be obtained have been given in previous publications (19) (22).

Two tests were performed on each patient. The first was an ordinary glucose tolerance curve; the second was the insulin-glucose test. The reason for performing the glucose tolerance test first was that in this way retardation of absorption, which may occur in nervous patients, could be detected. In cases showing such retardation the glucose tolerance test was repeated at intervals of two days until a rapid rise of hyperglycemia immediately after drinking the glucose revealed that absorption was normal and the patient habituated to the test. If this result was not achieved, experiments on this patient were discontinued. The insulin-glucose test was performed two or three days after the glucose tolerance test.

The dose of glucose given orally in both tests was 30 g. per sq. m. of body surface. This was dissolved in 400 c.c. of cold water and flavoured with essence of lemon and citric acid. The insulin used was Danish Leo insulin, 20 units per c.c. and the dose was 5 units per sq. m. of body surface. An accurately graduated tuberculin syringe fitted with a fine hyperdermic needle (No. 20) was used and the insulin was injected into the antecubital vein. In the insulin-glucose test the insulin was first injected and then immediately the patient drank the glucose solution.

Blood specimens. The blood sugar was estimated in 0.1 c.c. of capillary blood by the Hagedorn-Jensen method. The blood was drawn directly into the pipette from a puncture in the lobe of the warm ear.

Three samples were taken in the resting state and then either glucose alone or insulin followed by glucose were given. Samples were then taken at 5 min. and at 10 min. and at 10 min. intervals until 60 min.. In experiments directed to determine the total effect of injected insulin samples were taken for a further two hours at 15 min. intervals. The time of sampling was noted to the nearest quarter minute.

Urine sugar was estimated by Benedict's method.

Measurement of the results. In a previous communication an accurate method of measuring change in the blood sugar level in healthy subjects following either ingestion of glucose or injection of insulin was described (19). By this method the area traced out by the blood sugar curve either above or below the resting level is measured and the result expressed in milligramme-

minutes (mg. min.). Under unvarying conditions in one individual this area remains surprisingly constant and if the conditions are varied the effect of different conditions can be measured by comparing the change in the areas traced out. Under the same conditions healthy subjects show blood sugar curve areas which, whilst remaining approximately constant for one individual differ to some extent in different subjects, and, therefore, in considering the present results allowance must be made for this factor of individual variation (19). In the investigation into the effect of the insulin-glucose test in healthy subjects it was found necessary to terminate the observation at 60 min. (22) and as the results then obtained form the control for the present investigation, the experimental results in this paper refer, unless otherwise stated, to observations made during the first hour of the test.

The greatest difficulty in estimating the effect either of ingested glucose or injected insulin in diabetic patients arises from the fact that in different observations on such patients the resting blood sugar levels may differ. We have referred already to the work showing that in the fasting state the effect of insulin in depressing the blood sugar is proportional to the height of the resting blood sugar level (15) (17) (37) and in a previous communication we have also shown that a similar relationship exists between the degree of alimentary hyperglycæmia and the effect of insulin in suppressing it (22). Owing to the precautions taken to obtain a uniform state of control in each diabetic patient before the tests were commenced the fasting blood sugar level in the glucose tolerance test rarely varied much from the level of the insulin-glucose test obtained two days later and we have, therefore, presumed to chart the two curves thus obtained as starting from the same resting level. In experiments, however, in which the patient was taking different diets considerable variation in the resting blood sugar levels occurred and doubt may be felt as to the validity of comparing such curves. As will be seen later, however, these variations in the blood sugar level have been in a direction which has not altered our results qualitatively although it has minimised the extent of the measurable change.

For convenience of discussion the following abbreviated terms will be used. The area enclosed by the glucose tolerance blood sugar curve above the resting level will be called the Glucose Tolerance Area (G); the area enclosed between the glucose tolerance curve and the insulin-glucose curve, which results from the action of the injected insulin, will be called the Insulin Area (I); the area below the insulin-glucose curve, obtained by subtracting the insulin area from the glucose tolerance area will be called the Residual Area (R). This latter residual area can, of course, also be arrived at by measuring the area below the insulin-glucose curve; areas below the fasting blood sugar level being given a negative sign, areas above a positive sign, and the algebraic sum of the two being the residual area.

The I/G ratio (22) is the ratio between the insulin area (I) produced by a constant dose of insulin and the corresponding glucose tolerance area (G).

When the sensitivity to insulin remains constant, the effect of a constant dose of insulin in suppressing alimentary hyperglycæmia varies as the degree of hyperglycæmia upon which it acts, so that with diminishing degrees of alimentary hyperglycæmia the ratio, insulin area to glucose tolerance area (I/G area), remains constant or decreases slightly. A corollary of this observation is that if the I/G ratio diminishes whilst insulin sensitivity remains unchanged then the amount of injected insulin acting in the body is diminishing also. When the dietary conditions are constant, whatever the degree of alimentary hyperglycæmia, the I/G ration normally falls within a certain range of values (23); if it falls outside this range sensitivity to insulin is abnormal. When the sensitivity to insulin is varied, however, as when the dietary carbohydrate is varied, the I/G ratio for a constant dose of insulin varies also and in such a manner that the change in ratio parallels the change in sensitivity to insulin. In the same individual, therefore, the change in the I/G ratio in different circumstances is a measure of the change in sensitivity to insulin. This method of measuring insulin sensitivity is of especial value in diabetics in whom, owing to the wide variations in the fasting blood sugar level, direct measurements of change in insulin sensitivity by change in the insulin depression curve are not possible. It is convenient to present this short summary of the significance of the I/G ratio here as by means of this ratio the analysis of the results on diabetic patients has been greatly aided.

RESULTS.

The rate of insulin action in different diabetics.

In this section only the results obtained in the first 60 min. of the glucose tolerance and insulin glucose tests are discussed; the results thus concern the rate at which injected insulin comes into action and not its total effect. The results were obtained from 34 diabetic patients who were all controlled on diets ranging from 1,500 to 2,000 calories and containing from 150 g. to 250 g. of carbohydrate, the majority of the diets containing 175 g. of carbohydrate. Previously it has been shown that in healthy subjects the caloric value of the diet has no influence on either the sugar tolerance or insulin sensitivity but that these properties are influenced solely by the carbohydrate content of the diet. In the same investigation (19) it was also shown that increasing the dietary carbohydrate from 150 g. to 250 g. resulted in only an 8% improvement* in sensitivity to insulin or in glucose tolerance, and we have, therefore, permitted ourselves to compare curves from diabetics taking diets within this range and to consider that for our purpose the diet could be regarded as of constant composition.

* The increase in insulin sensitivity or in glucose tolerance in normal subjects caused by changing the dietary carbohydrate from 50 g. to 425 g. was taken as causing a 100% improvement in either insulin sensitivity or glucose tolerance, both properties improving in parallel (19).

The glucose tolerance curves and insulin-glucose curves from a normal subject and from two diabetic patients are given in Fig. 1 as example of the types of response obtained. It will be seen that in the normal subject and in the first diabetic patient the insulin-glucose curve falls at its commencement, that is the injected insulin comes into action immediately. The type of diabetic who shows this response to the insulin-glucose test we have called the insulin-sensitive type (21). The insulin-glucose curve from the second diabetic differs markedly from that of the normal subject and that of the first diabetic. Here the insulin-glucose curve rises from its start and shows

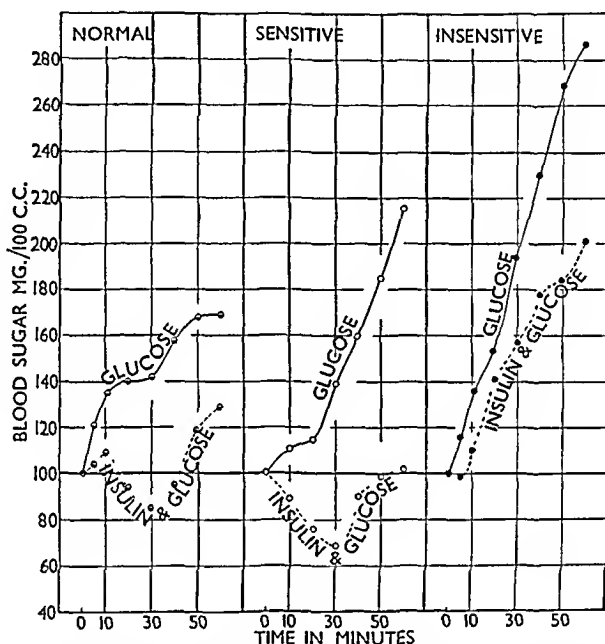


Fig. 1. Glucose tolerance and insulin-glucose curves from a normal subject, from an insulin-sensitive diabetic and from an insulin-insensitive diabetic. The composition of the diet was approximately the same in each case. The resting blood sugar levels in the normal subject were 91 and 102 mg./100 c.c., in the insulin-sensitive diabetic 240 and 247 mg./100 c.c., and in the insulin-insensitive diabetic 173 and 170 mg./100 c.c..

clearly that the injected insulin comes into action only slowly. Diabetics showing this response we have called the insulin-insensitive type (21). Inspection of these three curves will show the value of the I/G ratio in expressing the result. In the normal subject the value is 0.94, in the sensitive diabetic 1.27, in the insulin-insensitive diabetic 0.44. These values express clearly that in the insensitive patient the effect of injected insulin in suppressing alimentary hyperglycaemia is less than normal, the sensitivity to insulin is subnormal; whilst in the insulin-sensitive patient the effect of the injected insulin is not impaired, insulin sensitivity is not subnormal.

TABLE I.

	Glucose tolerance area to 60 min..			Insulin area to 60 min..			Residual area to 60 min..			I/G (60 min.).	
	No. of subjects	Average mg.min.	S.D.	No. of subjects	Average mg.min.	S.D.	No. of subjects	Average mg.min.	S.D.	No. of subjects	Average S.D.
*Normal subjects	10	3,929	—	13	3,353	—	10	189	—	10	0.97
Diabetics :—											
Insulin-sensitive	11	3,601	1075	11	4,534	1862	13	717	1116	11	1.34
Insulin-insensitive	13	5,656	1014	13	2,815	511	21	2898	928	13	0.31

S.D.—standard deviation of the observations.

In all cases the diet contained between 150 g. and 250 g. of carbohydrate.

The complete data obtained on them are not suitable for mathematical analysis. The reasons for this and for the selection of 10 subjects out of the total of 13 subjects, for inclusion in this table will be explained in the following paper (23).

It will be seen also that the I/G ratio provides a convenient method of denoting the type of response to the insulin-glucose test.

Of the thirty-four patients investigated 21 were insensitive and 13 were sensitive as judged by inspection of the curves.* The question now arises as to whether these two groups are clearly differentiated or merge into each other. This can be answered by charting all the I/G ratios for the group (Fig. 2). Both the glucose tolerance curve and insulin glucose curve being available in 13 insensitive patients and in 11 sensitive patients the I/G ratios in these subjects can be measured. The average value for I/G ratio in the insensitive diabetics is 0.51, standard deviation of the series, 0.11; for the sensitive diabetics 1.34,† standard deviation of the series, 0.39† (Table I). It will be seen that the average values are widely separated and that the relative smallness of the standard deviation of each average value indicates clearly that the two groups of figures are distinct and do not lie on a scale shading gradually from one extreme to the other (Fig. 2).

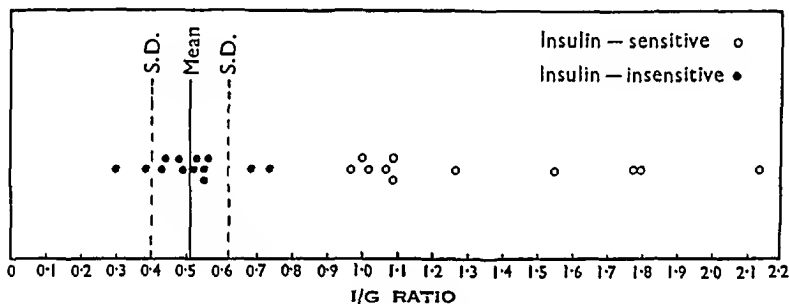


Fig. 2. Diagram showing the range of I/G ratio in insulin-sensitive (circles) and insulin-insensitive diabetics (discs). The continuous line indicates the mean value of the ratio and the broken lines on either side of it the standard deviation (S.D.) of the series of I/G ratio observed in the insulin-insensitive diabetics. It will be noted that the lowest value of the I/G ratio in the insulin-sensitive diabetics is more than four times the standard deviation of the insulin-insensitive diabetics distant from the mean value of the I/G ratio in the insulin-insensitive series. The composition of the diet was approximately the same for all patients.

The determination of the I/G ratio is a laborious procedure. A simpler test for the differentiation of diabetics was, therefore, sought and the significance of the residual area (R), that is the area below the insulin-glucose curve, was investigated from this point of view. The average value of the residual area in 21 insensitive diabetics is +2898 mg. min. with a standard

* These figures should not be taken as indicating the proportion of cases showing the two types of response in a representative diabetic population. Patients showing the insulin-sensitive response are often young and working, whilst those showing the insulin-insensitive response are often elderly and unemployed. As a consequence we have had less opportunity of investigating the former than the latter group.

† The mean and standard deviation of the series of I/G ratio in the insulin-sensitive diabetics are of questionable significance because in these patients the I/G ratio is influenced not only by sensitivity to insulin but also by the degree of insulin deficiency in the particular patients. For this reason these values for the sensitive patients have been omitted in Figs. 2 and 3.

deviation of the series of 928; the average value in 13 sensitive diabetics is —747 mg. min. with a standard deviation of the series of 1116. In Fig. 3 the values for the residual area in each patient in the insensitive and in the sensitive groups is charted. It will be seen that the values for the residual areas in the two groups do not overlap. The distinction, however, is not as sharp as when the I/G ratio is used and, although the mean values are far apart, the standard deviation of these values is not sufficiently small to disprove the suggestion that cases showing the two different responses are not part of one large group. The residual area is therefore not as valuable as the I/G ratio which differentiates the two types clearly.

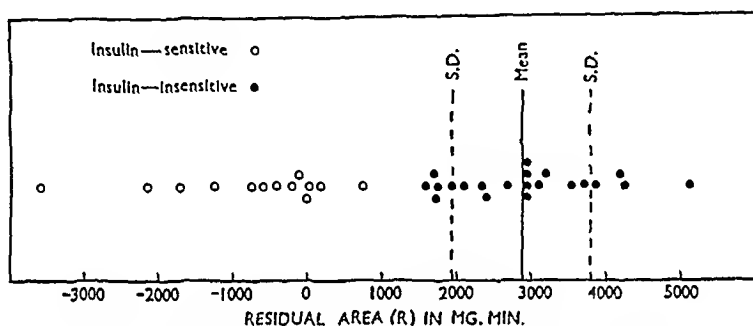


Fig. 3. Diagram showing the range of the residual area, R, in insulin-sensitive (circles) and insulin-insensitive diabetics (discs). The continuous line indicates the mean value of the residual area and the broken lines the standard deviation (S.D.) of the series of residual areas in the insulin-insensitive diabetics. The composition of the diet was approximately the same for all patients.

We conclude from these findings that two distinct groups of diabetics can be differentiated by the rate at which they react to injected insulin. In one there is a relative insensitivity to insulin which is shown by insulin coming into action only slowly, in the other the sensitivity to insulin is not impaired and insulin comes into action rapidly.

The total effect of insulin.

The question now arises whether the total effect of insulin in suppressing alimentary hyperglycaemia in both sensitive and insensitive diabetics is the same despite the difference in the initial rate at which it comes into action, or whether the initial impairment of insulin action shown by the insensitive patients persists so that the effect of the injected insulin when considered over a longer period still remains less than in the sensitive group. Data on this point are provided by the results of glucose tolerance and insulin-glucose tests extending over three hours.

It has previously been shown (22) that, *when insulin sensitivity is constant*, the effect of insulin in suppressing alimentary hyperglycaemia depends both upon the quantity of insulin injected and upon the degree of alimentary

hyperglycæmia on which it acts. The hyperglycæmia of the glucose tolerance test is of different degree in the first and in the second and in the third hours after glucose and, therefore, the absolute effect of a standard dose of insulin at these different times varies also. For this reason the insulin areas in the first hour cannot be compared with those of the second, and third hours after glucose ingestion. The I/G ratio, however, expresses insulin action relative to the degree of hyperglycæmia on which it acts and, therefore, a comparison of the I/G ratio during the first, the second and the third hours is permissible and, provided insulin sensitivity remains constant, will indicate whether the quantity of insulin acting is the same or whether it is increasing or decreasing. Under constant conditions of insulin sensitivity an unchanged I/G ratio indicates that the amount of insulin in action remains constant; if the I/G ratio falls then the amount of insulin has decreased; if the I/G ratio increases then the amount of insulin in action has increased.

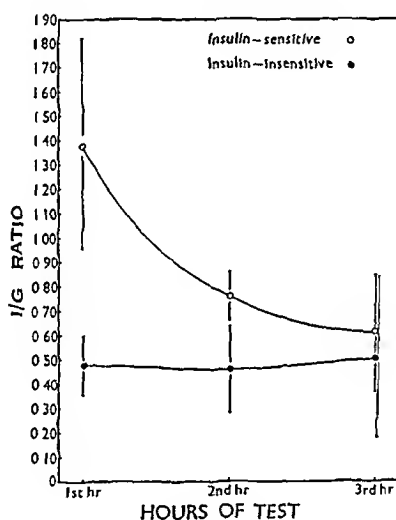


Fig. 4. Curves showing the change in the I/G ratio during the first, second and third hours of the tests in 6 insulin-sensitive diabetics (circles) and 6 insulin-insensitive diabetics (discs). The points represent mean values; the vertical lines the standard deviation of the observations. The diet was approximately the same in both series of diabetics. It will be seen that in the insulin-sensitive patients injected insulin comes into action rapidly and wanes quickly, whilst in the insulin-insensitive diabetic injected insulin maintains a steady low rate of action throughout the test.

In Fig. 4 curves are given which show the different type of change in the I/G ratio over the three hour period of the test in the sensitive and in the insensitive type of diabetic. It will be seen that in the curve for the sensitive patients the I/G ratio falls thus indicating the progressive decrease in insulin action. There are two possible explanations of this. First, the decrease in insulin action may be due to an increasing insensitivity to insulin. This suggestion is untenable because it is known that after alimentary hypergly-

cæmia insulin sensitivity is increased (16) (17). Second, the falling off in insulin action may be due to the diminution in the quantity of insulin acting because of utilisation of the injected insulin. This latter explanation is the one which is probable.

In the curve of the insulin-insensitive diabetics on the other hand the I/G ratios do not diminish but remain approximately constant. Consideration of this result suggests that the impairment of insulin action in this type of diabetic is not due to excretion or to destruction of a proportion of the injected insulin. If either of these explanations were correct then the surviving insulin would presumably have been utilised at a similar rate as in the sensitive patients with the result that the I/G ratios, whilst of smaller magnitude, would have formed a similar diminishing series of values. The constancy of the I/G ratio throughout the tests appears to indicate that in insensitive diabetics a condition exists which, without destroying insulin, retards its action so that its effect is only manifested slowly. Such a condition might result from either the presence of a retarding inhibitor of insulin action or, to deficiency of an activator or coenzyme of insulin (16).

It would have been desirable to present here the sequence of changes in the I/G ratio over a period of three hours obtained on healthy subjects but for technical reasons this is not possible. In healthy subjects the insulin injected in the insulin glucose test depresses the blood sugar to such low levels that a compensatory liberation of sugar from the liver into the blood is evoked. This inflow of endogenous sugar reinforces the inflow of sugar from the gut so that often the insulin-glucose curve in the second hour rises higher than the corresponding glucose curve and the insulin area becomes negative in value. The significance of the I/G ratio calculated from such negative values is doubtful (22).

The total effect of insulin in the two types of diabetics over the three hours can be measured by the I/G ratio of the total areas over the time (insulin area to three hours/glucose tolerance area to three hours). For the insulin-sensitive diabetics the average I/G ratio is 0.79 with a standard deviation of 0.11; for the insulin-insensitive diabetics 0.46 with a standard deviation of 0.18. It is possible that if the period of observation had been extended beyond three hours they would have approached each other even more closely. It is, therefore, suggested that the total effect of a dose of insulin in suppressing alimentary hyperglycæmia is approximately the same in both types of diabetic, but that in the sensitive patient, and normal subject, the injected insulin comes into action rapidly and is quickly exhausted whilst in the insensitive type the rate at which it comes into action is retarded so that its full effect requires a longer period to become manifest.

Insulin sensitivity in Cushing's syndrome: the influence of the fasting blood sugar level on the glucose tolerance and insulin-glucose test.

The insulin sensitivity in two cases of Cushing's syndrome with diabetes has been tested and we are grateful to Dr. Charles Miller for permission to

TABLE II.

Date.	Dietary carbo- hydrate. g..	Insulin require- ment.	Glycosuria.	Average fasting blood sugar mg./100c.c..	Glucose tolerance area to 60 min. mg. min..	Insulin area to 60 min. mg. min..	I/G ratio.			I/G ratio over 3 hours.
							1st hr.	2nd hr.	3rd hr.	
Nov. 1937	177	25	++	142	6960	2700	0.39	0.23	0.37	0.33
Sept. 1938	177	0	trace	110	6660	4440	0.67	0.36	0.20	0.41

Data from a case of Cushing's syndrome before (Nov. 1937) and after (Sept. 1938) X-ray irradiation of the pituitary region.

investigate these cases. Both cases were found to have diabetes of the insulin-insensitive type. De Wesselow and Griffiths (7) mention that their modification of the insulin-glucose test revealed a high degree of insulin insensitivity in a case of Cushing's syndrome and Flaum (11) has reported a similar finding in cases of acromegaly.

Case 1, a woman aged 43, was only discovered to have diabetes when a routine blood sugar curve was carried out. This test revealed diabetes of a mild degree but the insulin glucose test showed a minor degree of insulin insensitivity, the I/G ratio to 60 min. being 0.69. This case is of interest as showing that even though diabetes were mild it was of the insulin-insensitive type.

Case 2, a woman, aged 40 years, had had mild diabetic symptoms some 6 months previously but these had been absent since and during her admission to hospital. Her fasting blood sugar level, when receiving 25 units of insulin daily, was raised to the average of 142 mg./100 c.c. and her glucose tolerance curve was frankly diabetic. Insensitivity to insulin was revealed by an I/G ratio to 60 min. of 0.39 and by the persistence of the I/G ratio at this low figure in the first, second and third hours of the test (Table II). The patient was receiving a diet containing 177 g. of carbohydrate and 25 units of insulin daily. Despite this she continued to pass moderate amounts of sugar in the urine. X-ray irradiation of the pituitary region was carried out by Dr. Gwen Hilton and the improvement in the patient's condition appeared during the treatment and persisted afterwards. She rapidly became sugar free and the insulin dosage was reduced until it was omitted entirely. With no insulin the patient passed an occasional trace of sugar; on 5 units daily she remained sugar free. The systolic blood pressure fell concurrently from 175 mm. Hg to 120 mm. Hg. A second series of tests was performed 10 months after the first. The fasting blood sugar now averaged 110 mg./100 c.c. but despite this the area of her glucose tolerance curve was approximately the same. Her sensitivity to insulin had, however, increased so that the 60 mm. I/G ratio had risen to 0.67; and the fall of the I/G ratio from the first to the third hour of the test suggested that the total response to insulin was approximating to the insulin-sensitive type. In Table II the results of the two series of tests are summarised and in Fig. 5 the glucose-tolerance and insulin-glucose tests before and after X-ray irradiation of the pituitary region are shown.

This latter case raised two points of interest. First, with the use of measures directed against the pituitary, the insulin insensitivity diminished. This point will be discussed further when the relationship of the pituitary gland to insulin insensitivity is considered. The second point concerns the glucose tolerance area which in this patient was the same on both occasions despite the fall in the fasting blood sugar level. Now when insulin sensitivity remains constant it is known that the higher the blood sugar, whether fasting or after ingestion of glucose, the greater is the absolute effect of a standard dose of insulin. The fact that in this patient the glucose tolerance curve was no greater on the second occasion, when the fasting blood sugar was low, than

on the first occasion when the fasting blood sugar was high, indicates that on the second occasion either more pancreatic insulin was available to restrain the rise of blood sugar, or that the sensitivity to insulin had increased so that the insulin secreted acted more effectively. While there is no evidence to support the first alternative, evidence for the second has already been provided. We, therefore, regard this case as supporting the impression we have gained from other work that if on two occasions the glucose tolerance

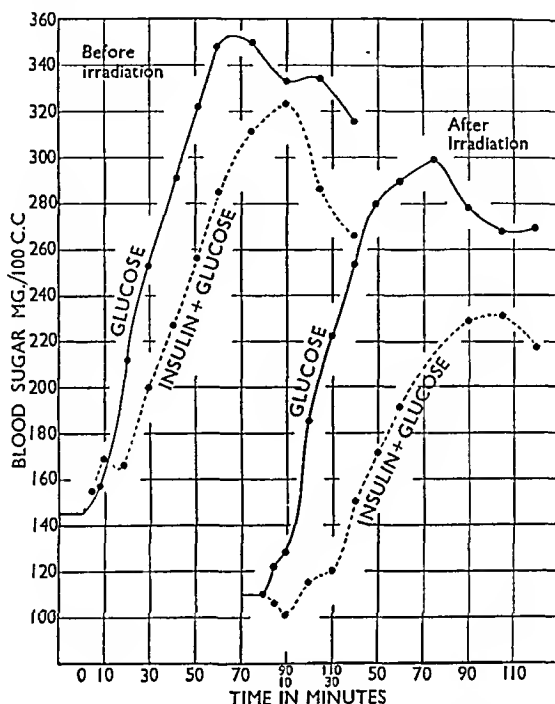


Fig. 5. Glucose tolerance and insulin-glucose curves obtained before and after X-ray irradiation of the pituitary region in a case with Cushing's syndrome. The improvement in the patient's condition is shown by the fall in the resting blood sugar level and the increased response to the insulin injected in the insulin-glucose test. Composition of the diet before both tests, carbohydrate 177 g., protein 80 g., fat 81 g..

curves are of the same area, and yet the initial blood sugar levels are different, then the glucose tolerance curve commencing at a high blood sugar level indicates a lower degree of tolerance than that commencing at a lower level. We also think that the initial blood sugar level influences similarly both the insulin-glucose curve and consequently the insulin area. These considerations are directly relevant to the data in the next section.

The effect of change in the composition of the diet.

In a previous paper (19) it has been shown that increasing the carbohydrate content of the diet improves both the sensitivity of the body

to insulin and the sugar tolerance in the healthy subject. If the explanations we have given of the two types of diabetes mellitus be correct then it might be expected that the diabetic, whose disease is due to lack of insulin, would react to increase of dietary carbohydrate in a manner different from that of the diabetic, whose disease is due to insensitivity to insulin. This has been found to be the case.

The effect of changing the dietary carbohydrate was studied on 6 sensitive and 9 insensitive patients, 14 experiments being performed on the first group and 23 on the second. Alterations in the amount of sugar passed in the urine during 24 hours as the result of increasing the dietary carbohydrate was investigated in each case. The patient was first given the diet containing

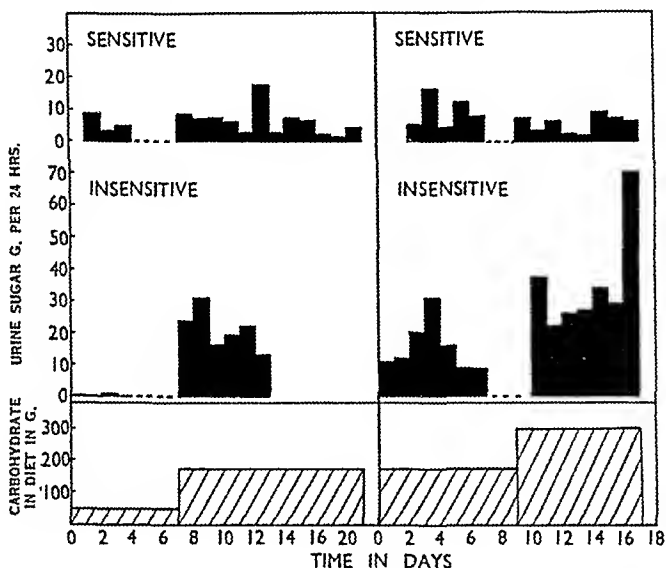


Fig. 6. Chart showing the effect of increasing the carbohydrate content of the diet upon the daily excretion of sugar in two insulin-sensitive and two insulin-insensitive diabetics. The dotted base line corresponding to the end of the first dietary period indicates the days during which glucose tolerance and insulin-glucose tests were being carried out and when, as a consequence, the usual rate of excretion of sugar in the urine was disturbed. The daily dosage of insulin in each case remained unchanged throughout the period of observation.

least carbohydrate and, if necessary, insulin was given until he was excreting only traces of sugar in the urine. The excretion of sugar in the urine was then measured daily during the next week and if the total daily excretion remained approximately constant the patient was considered to be satisfactorily "balanced." The appropriate blood sugar tests were then performed. During this test period no measurements were made on the urinary excretion as the starvation before and ingestion of glucose during the tests rendered such measurements fallacious. After the tests were completed the higher carbohydrate diet was given but the insulin dosage was

maintained unaltered. It was found that in the insensitive diabetics, increasing the dietary carbohydrate, resulted in an increase in the quantity of sugar excreted in the urine. Typical examples are given in Fig. 6. It is noteworthy, however, that the increase in glycosuria never equalled the increase in dietary carbohydrate. In the case of the sensitive diabetics, increase of dietary carbohydrate resulted either in no increase or in a decrease of glycosuria. It can, therefore, be said that increasing the dietary carbohydrate causes no increase in glycosuria in the sensitive case but causes considerable increase in glycosuria in the insensitive diabetic.

In the method of control of diabetic treatment by blood sugar estimation it has been customary to estimate the influence of any particular therapeutic measure by its effect upon the fasting blood sugar level. If a particular treatment caused this level to rise then the influence of the treatment was considered to be deleterious, if it caused the level to fall its influence was concluded to be favourable. In Table III are given the changes in the average fasting blood sugar level of a series of diabetics of both types when taking diets containing different amounts of carbohydrate. It will be seen that when the carbohydrate in the diet is raised there is a slight tendency for the fasting blood sugar level to rise in the insensitive cases but that it remains unaltered or even falls in the sensitive diabetics.

A similar difference in effect in the two types of diabetics of increase of dietary carbohydrate can be demonstrated by the change in the sugar tolerance curve. Examples of the difference in response are given in Fig. 7. This shows that in the insensitive case increase of dietary carbohydrate results in impairment of the sugar tolerance, whilst in the sensitive case it causes improvement of sugar tolerance. The results are not always as clear cut as this possibly because of the influence of the change in resting blood sugar level. The tendency of the glucose tolerance curves is, however, clear. In the sensitive diabetic the curves tend to decrease with each increment of dietary carbohydrate; in the insensitive diabetic the curves increase or remain of constant area. The increased significance of such changes when considered in relation to change in the initial blood sugar level has been suggested in the previous section.

The effect of dietary changes on the insulin-glucose curve parallels the change in the glucose tolerance curve (Fig. 7). Increase in dietary carbohydrate in sensitive diabetics resulted in a lower insulin-glucose curve and in insensitive diabetics in a higher insulin glucose curve. In two further experiments patients who were apparently insensitive on the diet containing 50 g. of carbohydrate became sensitive on the diet containing 175 g. of carbohydrate. We are inclined to regard the insulin-insensitive curve in these cases as an artefact. Both patients were very nervous before the first test and it is possible that fright, by calling forth a secretion of adrenaline, resulted in an antagonisation of the action of the injected insulin so that spurious insensitive curves were produced. In none of the other fourteen experiments on insensitive diabetics was there any suggestion that increase

in dietary carbohydrate changed the insensitive type of curve to the sensitive type. It would appear that, provided the diabetes is under full control, subsequent change in the carbohydrate content of the diet does not result in change in the type of response to the insulin-glucose test.

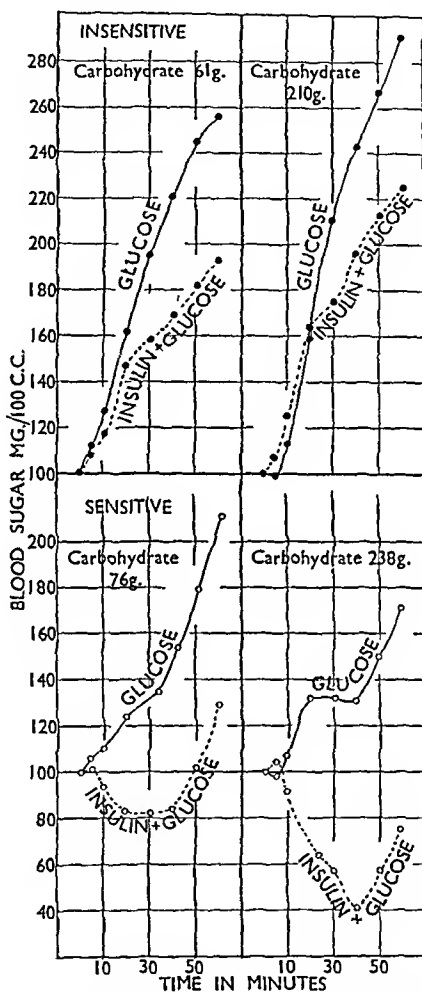


Fig. 7. Chart showing the effect in an insulin-sensitive and in an insulin-insensitive diabetic of increasing the carbohydrate content of the diet upon the glucose tolerance and insulin-glucose tests. In the sensitive patient the dietary carbohydrate was increased from 76 g. to 238 g.; in the insensitive patient from 61 g. to 210 g.. The caloric value of the diets was the same in all cases. For convenience of drawing the curves have all been made to start from 100 mg./100 c.c.. The average levels in each case were insensitive patient, low carbohydrate diet 173 mg./100 c.c., high carbohydrate diet 143 mg./100 c.c.; sensitive patient low carbohydrate diet 135 mg./100 c.c., high carbohydrate diet 140 mg./100 c.c..

In view of the correlation that has been shown to exist between glucose tolerance and insulin sensitivity (17) (18) (19) it is natural to expect that the

differing changes in sugar tolerance in response to change of dietary carbohydrate shown by each of the two types of diabetics would be correlated with a similar change in insulin sensitivity. We have previously shown that, when the dose of insulin is constant, change in sensitivity to insulin in normal subjects is revealed by change in the I/G ratio to 60 min. and we have attempted to use this method of approach in the investigation of change in insulin sensitivity in diabetics. Increase of dietary carbohydrate causes increase in insulin sensitivity in sensitive diabetics, whilst in insensitive diabetics, it either has no effect on sensitivity or causes only a relatively slight improvement (Fig. 7). We are not yet convinced of the existence of the slight improvement in this latter type of case.

The effect of dietary changes in the two types of diabetics may be summarised. In the case of the sensitive diabetic increase of the carbohydrate content of the diet causes no increase in glycosuria, no rise in the fasting blood sugar level, but produces improvement of sugar tolerance and of sensitivity to insulin. In the case of the insensitive diabetic increase of dietary carbohydrate causes increase in glycosuria, a tendency to higher fasting blood sugar levels, impairment of sugar tolerance and little or possibly no increase in sensitivity to insulin.

Correlation of the type of response to the insulin-glucose test with clinical data.

The clinical data in 14 diabetics showing the insulin-sensitive response and in 22 diabetics showing the insulin-insensitive type of response are given to Table IV. It will be seen that clinically the cases showing the different types of response to the insulin-glucose test are not sharply differentiated.

As regards the sex distribution, family history of diabetes, incidence of retinitis, and insulin requirements the two groups do not differ, but as regards the other characteristics there is a definite tendency to their predominance in either one or the other group of patients. On the whole the sensitive diabetics tend to be younger, thin, to have a normal blood pressure and to have healthy arteries and in them the disease is, as a rule, of sudden and severe onset so that they speedily seek out medical treatment. The insensitive diabetics on the other hand tend to be older, obese, to have hypertension and frequently to exhibit pronounced arteriosclerosis and in these patients the onset of the disease is insidious so that the taking of medical advice is long delayed. The more conspicuous differences shown in Table IV are in respect of the infrequency of obesity and the frequency of acute onset of the disease in the sensitive patients.

Two further clinical points are noteworthy. As a rule sensitive diabetics easily develop ketosis and react to a slight excess of insulin with a hypoglycaemic attack. Insensitive diabetics rarely develop ketosis and can usually tolerate considerable over-dosage of insulin without showing symptoms of hypoglycaemia. It will thus be seen that, whilst no definite correlation between the clinical data and the response to the insulin-glucose test can be

TABLE IV.

Clinical data of diabetics giving the two different responses to the insulin-glucose test.

INSULIN-SENSITIVE RESPONSE TO INSULIN-GLUCOSE TEST.

No.	1	2	3	4	*5	6	7	8	9	10	11	12	13	14
Sex	M.	M.	F.	M.	M.	F.	F.	M.	M.	F.	M.	M.	M.	M.
Age	27	37	64	34	23	21	34	57	23	58	63	24	62	19
Family history of diabetes	+	o	o	o	o	o	o	o	o	+	o	o	o	o
Acute onset	+	+	+	+	+	+	+	+	+	+	o	+	o	+
†Duration before test	1 m.	1 y.	1½ y.	3 w.	1 y.	1 y.	3 m.	6 y.	6 y.	3 m.	?	2 w.	?	2 w.
Obesity	o	o	o	o	o	o	o	o	o	o	o	o	+	o
B.P.	109 65	110 80	195 80	108 80	90 50	108 80	142 80	145 80	105 85	145 70	—	105 60	196 118	105 75
Arteriosclerosis	o	o	+	o	o	o	o	+	o	+	+	o	o	o
Retinitis	o	o	+	o	o	o	o	+	+	o	o	o	+	o
Insulin requirements	20	20	20	25	64	110	35	30	55	35	o	40	20	28

INSULIN-INSENSITIVE RESPONSE TO INSULIN-GLUCOSE TEST.

No.	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	*34	35	36
Sex	M.	F.	F.	F.	F.	F.	F.	F.	F.	M.	F.	F.	F.	M.	F.	F.	M.	M.	F.	M.	M.	F.
Age	67	57	40	55	71	31	65	58	50	63	29	65	59	53	56	68	40	56	43	55	30	59
Family history of diabetes	o	o	o	o	+	o	o	+	o	o	+	o	o	+	o	o	o	o	o	o	o	o
Acute onset	o	o	o	+	o	+	o	o	o	o	+	+	+	+	+	+	+	o	o	o	?	+
†Duration before test	4 y.	10 y.	?	?	1 y.	1½ y.	?	?	2 y.	18 y.	2 m.	5 y.	6 y.	2 m.	6 m.	1 y.	15 y.	2 y.	?	20 y.	1 y.	4 y.
Obesity	o	+	+	o	+	o	+	o	o	+	o	o	+	o	+	+	o	o	o	o	o	+
B.P.	150	107	170	125	205	120	230	164	114	196	108	232	198	150	164	290	153	130	160	165	105	188
	80	85	95	90	100	90	120	85	45	96	70	108	90	80	70	140	80		95	78	70	110
Arteriosclerosis	+	+	o	o	+	o	+	+	o	+	o	+	+	o	+	+	o	+	o	+	o	+
Retinitis	o	+	o	+	o	o	o	o	o	o	o	+	o	o	o	o	+	o	o	+	o	o
Insulin requirements	0	40	25	35	30	25	0	25	45	50	50	40	35	25	65	40	25	90	0	15	75	55

* Patient not tested on standard diet, therefore, his result is not included in the calculations.

† w = weeks; m = months; y = years. The duration of the illness is measured from the onset of symptoms. It is probable that in many of the insulin-insensitive cases the disease has existed for years without exciting the patient's concern, until the occurrence of a mild infection intensified the diabetes and thus induced indisputable symptoms. In such cases the duration of the disease is naturally dated from this episode.

established, yet the usual patient who gives either one or other response is of a definite clinical type. These two clinical types have long been distinguished in the minds of clinicians (9) (10) and possibly the strongest consideration in effecting this distinction has been the knowledge that the typical patient giving the insulin sensitive response dies in a relatively short time if the disease is untreated, whilst the typical patient who gives the insulin insensitive response may neglect the care of his disease for years and yet survive in tolerable health.

The finding that insensitive diabetics do not require considerably more insulin than do sensitive cases is at first sight surprising in view of the observed greater initial effect of injected insulin in the latter patients. But it will be remembered that the total effect of insulin in the two types of diabetics when measured over a period of three hours tends to approximate and that it is, therefore, possible that over a longer period, such as twenty four hours, the total effect of insulin may be the same in both types of cases. If this be so then a great difference in the daily insulin requirements between a group of sensitive and a group of insensitive patients would not be expected.

The observation that in many of the insensitive diabetics the disease has existed for many years before the patient came under medical treatment, and that in sensitive diabetics it had usually only been present for a short time (Table IV) raises the question as to whether the disease at its onset is always of the sensitive type and that in the course of years it may change to the insensitive type. In respect of this consideration it is important that the insensitive type of diabetes may be reported as of sudden onset. Insensitive Case 17 (Table IV) is of particular interest as one year previously to the onset of diabetic symptoms the urine was known to have been free of sugar. In two of the sensitive diabetics, Cases 8 and 9 (Table V) diabetes had been present for the 6 years before our observations were made, and in both cases the patients had exercised only the minimum control over their disease in this period. When one considers, however, that in some of the insensitive diabetics the disease has been present for 18 or 20 years before our observations were made it will be seen that the presence of an insulin-sensitive response at the end of 6 years is insufficient evidence upon which to suggest the permanency of the sensitive type of response. A definite answer is desired on this point for the following reason. It has been suggested by one of us that long continued ingestion of low carbohydrate diets might permanently impair insulin sensitivity and so produce diabetes mellitus (20). If this suggestion be correct then the treatment of a sensitive diabetic by means of a low carbohydrate diet, or the imperfect control of such a diabetic so that by reason of gross glycosuria the effect of a liberal carbohydrate allowance is stultified, might change a sensitive to an insensitive case. At present all that can be said is that a diabetic may be insulin-insensitive from the onset but that there is no evidence as yet that after many years an insulin-sensitive diabetic will still be insulin-sensitive.

DISCUSSION.

The results which have been presented in this paper can now be conveniently summarised. By means of the insulin-glucose test performed under the conditions described diabetics can be differentiated into two types ; one type is characterised by normal sensitivity to insulin, and the other by impaired sensitivity to insulin. When, however, the effect of insulin is measured over several hours the total effect of insulin, relative to the degree of alimentary hyperglycæmia on which it acts, tends to approximate to a similar value in both types of case. This suggests that the essential difference between the two types lies in the different rates at which insulin comes into action rather than in its total effect. Although it is probable that cases of an intermediate character exist, these are rare, so that the differentiation between the two types is definite and a frequency curve shows two separate groups of cases and not a gradual transition from one extreme to the other. The two types of diabetics react differently to dietary change. The sensitive diabetic reacts favourably to increase of dietary carbohydrate, glycosuria is not increased, the fasting blood sugar level does not rise, the glucose tolerance test either improves or remains constant, the insulin-glucose test improves, and the sensitivity to insulin increases. The insensitive diabetic on the other hand responds unfavourably to increase of dietary carbohydrate, glycosuria increases, the fasting blood sugar level tends to rise, the glucose tolerance and insulin-glucose tests either show impairment or remain constant and the sensitivity to insulin either does not change or improves only slightly. Insensitive diabetics are not changed into sensitive diabetics by increase of dietary carbohydrate within the range of diets tested. Clinically patients giving the different types of response to the insulin-glucose test are not sharply differentiated, but the usual patient in either group conforms to a recognizable clinical type. The sensitive diabetic tends to be young and thin. He seeks medical advice early because of the severe and acute onset of the disease ; his blood pressure is normal, his arteries healthy ; if untreated he rapidly develops ketosis ; slight excess of insulin speedily results in a hypoglycæmic attack. The insulin-insensitive diabetic on the other hand, tends to be older and obese, his disease is of a mild and insidious onset ; he frequently has hypertension and arteriosclerosis ; ketosis develops rarely and slowly ; he will tolerate insulin in considerable excess of his requirements without experiencing symptoms of hypoglycæmia.

The insulin-glucose test was described and its results in the early experiments were reported in a preliminary communication by one of us (21) three years ago. Further experience which is now reported has confirmed the conclusions regarding the significance of the results which previously were put forward only tentatively. Results and conclusions conflicting with ours have, however, been reported by de Wesselow and Griffiths (7). These workers, whilst confirming the existence of two different types of response to a 60 min. insulin-glucose test, state that the test affords no

evidence for the existence of two different types of diabetics and that instances of insulin sensitivity and insensitivity merge into a larger group showing an approximate normal response. They further report that it is possible by increasing the dietary carbohydrate and giving adequate supplies of insulin to convert an insulin-insensitive to an insulin-sensitive response. The insulin-glucose test used by these workers was not the test as originally described and it is to the modifying of the original test that the discrepancies between our results and theirs can be traced. In the test as modified by them only three blood sugar estimations are made, one in the fasting state and the others at thirty and sixty minutes respectively after administration of 50 g. of glucose and five units of insulin. The maximum depression of the blood sugar in the insulin-glucose test in the majority of normal subjects and sensitive diabetics occurs within 30 minutes of the insulin injection and consequently the modified test fails to detect all sensitive cases save those in which the sensitivity to insulin is so extreme that a marked depression of the blood sugar persists beyond 30 minutes. As a result the majority of sensitive diabetics are classified as moderately insulin insensitive and constitute a group intermediate between the extremes of sensitivity and insensitivity to insulin. It will be seen that if, as a result of using the modified test, a sensitive diabetic is wrongly classified as insensitive then increase of dietary carbohydrate, by improving sensitivity to insulin, may result in the next modified insulin-glucose test showing an insulin sensitive response and thus give the erroneous impression that the previous insulin insensitive response has been changed to one that is insulin sensitive. We thus do not consider that the modification of the insulin-glucose test used by de Wesselow and Griffiths differentiates with sufficient accuracy sensitive from insensitive diabetics and we are therefore unable to accept the conclusions based upon it.

Since the introduction of insulin there has been steadily accumulating a body of clinical experience which suggests that insulin does not act with equal efficiency in all cases of diabetics. On the basis of such experience Falta and his school (9) (10) have postulated the existence of two types of diabetes, the insulin sensitive and the insulin resistant type. The two main tests which they have used to distinguish these types have been the determination of the "glucose equivalent of insulin," and the determination of the "insulin tolerance." In the former test a standard dietary is given until the glycosuria is constant in amount, and then the dose of insulin required to abolish this glycosuria is determined. It is found that in sensitive diabetics relatively few units suffice to free the urine of sugar, and therefore their glucose equivalent of insulin is high, whilst in insulin resistant patients many units of insulin are required to remove the glycosuria and the equivalent is low. In the second test the patient is first rendered sugar free and then on successive days successively larger doses of insulin are given until a hypoglycæmic attack is produced. In the sensitive diabetics a few units extra of insulin result in a hypoglycæmic attack; in

the resistant diabetics a large excess of insulin is tolerated without an attack developing. Corresponding to these two types of patients two clinical groups of diabetics can be recognized and these two groups coincide with the two typical clinical groups which give different responses to the insulin-glucose test. The typical insulin sensitive patients distinguished by either test have the same characteristics; Falta's typical insulin resistant cases and our typical insulin insensitive cases are identical. And either by Falta's test or by the insulin-glucose test cases are frequently encountered which clinically are atypical. It therefore appears that the measuring of the glucose equivalent of insulin, the insulin tolerance test and the insulin-glucose test each distinguish the same two types of diabetes.

A word on the terminology of the two types of diabetics is appropriate. We came to use the term insulin insensitive because our investigations on these patients arose from work on the sensitivity to insulin of normal subjects and we have continued to use this term in preference to the term insulin resistant for two reasons. First, the term insulin resistant has already been used in a special sense to describe those cases of diabetes in which doses of insulin of the order of 500 to 1,000 units daily are unable to control the disease (2) (8) (34) (35) (38). Secondly, the mechanism of insensitivity to insulin is not yet established and the simple descriptive term insulin insensitivity thus appears preferable to the term insulin resistant with its implication that the underlying mechanism is an antagonization of insulin action.

MacBryde (29) has reported the effect of dietary change on the two types of diabetics differentiated by Falta's tests. His results are the reverse of ours and this discrepancy has retarded the acceptance of both Falta's and our work in this field (39). MacBryde has concluded that insulin resistant diabetics are improved by increase of carbohydrate whilst insulin sensitive cases are made worse. It is difficult to see from his published results how he arrived at the first of these conclusions. On examining his figures for the insulin resistant cases it can be seen that in every case increase of dietary carbohydrate results in increase of glycosuria and rise in the blood sugar level, findings which suggest that the increase in dietary carbohydrate has made the disease worse. In the case of the second conclusion the discrepancy seems to arise from the shortness of the observation period. When a *normal* subject who has been taking a low carbohydrate diet, is given a high carbohydrate diet it is frequently observed that glycosuria occurs during the first day on the new regime, and that thereafter improvement of sugar tolerance sets in rapidly. This effect also occurs in diabetics. When insulin-sensitive patients receive a higher carbohydrate diet increase in glycosuria may occur during the first two or three days and then improvement sets in rapidly. The same increase happens in insulin insensitive patients but in their case improvement does not follow (Fig. 7). It is easy to see that if the observation period on the high carbohydrate diet is confined to 3 or 4 days, as was the case in the majority of MacBryde's experiments, then the

impression would be gained that increase of dietary carbohydrate has a deleterious effect upon the carbohydrate tolerance of sensitive diabetics. We therefore consider that MacBryde's results on sensitive patients are open to doubt and that his results on insulin resistant cases accord with our findings in insensitive patients.

The effect of increasing the carbohydrate content of the diet upon the sensitivity of diabetics to insulin has been studied by Card (5). The observations were made upon young diabetics and therefore presumably the majority were of the sensitive type. Using as his test the insulin depression curve after intravenous injection of 5 units of insulin, Card came to the conclusion that increase of dietary carbohydrate from 100 g. to 200 g. daily had no effect upon the diabetic's sensitivity to insulin. The change in sensitivity to insulin of normal subject resulting from this increase of carbohydrate is 16% ; the experimental error of the insulin depression test as computed by Card is 9%. It is therefore not surprising that no increase in insulin sensitivity was detected.

The question of the effect of high and of low carbohydrate diets on the two types of diabetics has an important clinical bearing. With the introduction of higher carbohydrate diets into the therapeutics of diabetes a controversy arose, one school of clinicians maintaining that such diets increased glycosuria and the fasting level of the blood sugar, and necessitated a large increase in insulin dosage, and the other that no increase of glycosuria or blood sugar level occurred and that the insulin requirements either remained constant or decreased. Our results offer a means of reconciling these two divergent views, for it appears that the result of the higher carbohydrate diets depends upon the type of diabetic to which they are given ; in the sensitive patient the result is favourable, in the insensitive patient unfavourable. We would stress, however, that an insensitive patient's difficulty in dealing with a higher carbohydrate diet is no evidence that he does not need such a liberal supply of carbohydrate.

Having now discussed the evidence for the differentiation of cases of diabetes mellitus into two types by means of the response to the insulin-glucose test and the reaction of the two types to dietary change, consideration can be given to the mechanism underlying this difference. On examining the glucose tolerance and the corresponding insulin-glucose curves from sensitive and insensitive diabetics an explanation of the difference suggests itself at once (Fig. 1). In the sensitive type the impaired glucose tolerance is largely corrected by administration of insulin ; in the insensitive type the sensitivity to insulin is impaired and the abnormal glucose tolerance curve is not corrected by injection of insulin. The suggestion follows that in the sensitive patient the disease is due to lack of insulin whilst in the insensitive patient the disease is due, not to lack of insulin, but to insensitivity to insulin (21). The results reported in this paper support this suggestion.

On examination of the values for the insulin areas in normal subject,

and in sensitive and in insensitive diabetics it will be seen that in the normal subjects and in the insensitive diabetics the absolute values for these areas are approximately the same but that in the insulin sensitive diabetics the areas are much larger (Table I, Fig. 9). It thus appears that the sensitive diabetic is more sensitive to insulin than, not only the insensitive diabetic, but also the normal subject. Such an apparently increased sensitivity to insulin in some diabetics has also been noted by de Wesselow and Griffiths (7). This point is further brought out by the I/G ratios; the average I/G ratio in the normal subject is 0.97, in the insensitive patient 0.51, in the insulin-sensitive patient 1.34 (Table I). The question now arises, is there in the sensitive diabetic a true increase in sensitivity to insulin or is the increased effect of insulin in these subjects explicable in another way. To answer this question reference must be made to previous work on the relationship between hyperglycæmia and insulin action (22).

In Fig. 8 is reproduced the curve showing the relationship, when insulin sensitivity remains constant, between the residual area (area below insulin-glucose curve) and the dose of insulin. This curve shows that equal increments of insulin produce progressively smaller effects in suppressing alimentary hyperglycæmia. If we assume that the relationship expressed by the curve holds beyond its limits, then continuing the curve below the point corresponding to 0 units expresses the relationship when the patient is diabetic from lack of insulin. It will be seen that the effect of a standard dose of insulin will be greater in such a diabetic patient than in a normal subject and further that, with constant sensitivity to insulin the more diabetic the patient is from lack of insulin the greater will be the effect of the standard dose of insulin in suppressing alimentary hyperglycæmia. Partly for this reason, and partly because of the effect of the elevated fasting blood sugar levels, the absolute effect of a standard dose of insulin tends to be greater in pancreatic diabetes than in healthy subjects. In normal subjects the effect of insulin relative to the degree of hyperglycæmia on which it acts is expressed by the I/G ratio and it has been found that when sensitivity to insulin is constant this ratio either remains constant or increases slightly as the degree of hyperglycæmia increases. If the latter finding expresses the correct relationship it follows that as a subject becomes more diabetic from lack of insulin the insulin area increases slightly more rapidly than the glucose tolerance area. It is on these lines that we explain the large absolute effect of a standard dose of insulin upon the alimentary hyperglycæmia and the increased I/G ratio in sensitive diabetics. It thus appears that *the insulin-sensitive diabetic reacts to insulin in a manner that an individual diabetic from lack of insulin, and yet normally sensitive to insulin, would be expected to react.*

In the insensitive diabetic, as we have seen, insulin seems to come into action slowly (Fig. 1). On examining the insulin areas it is found that they are of approximately the same size in insensitive diabetes (mean 2815 mg. min.) as in normal subjects (mean 3353 mg. min.). When the glucose tolerance

area is plotted against the corresponding insulin area (Fig. 9), it is found that in the insensitive diabetic the insulin area no longer increases with the glucose tolerance area as it does in normal subjects (22), and to some extent in sensitive diabetics. It remains relatively constant. This indicates that in insensitive diabetics the normal effect of increased hyperglycæmia in increasing insulin action is offset by an impairment of insulin action itself so that the absolute effect of insulin remains at approximately normal values. It has been shown previously (22) when a subject passes from a high to a low carbohydrate diet that, despite a gradually increasing glucose tolerance area, the insulin area does not increase but remains constant. This result has been attributed to a gradual falling off in the sensitivity to insulin which neutralises the potential effect of the increased alimentary

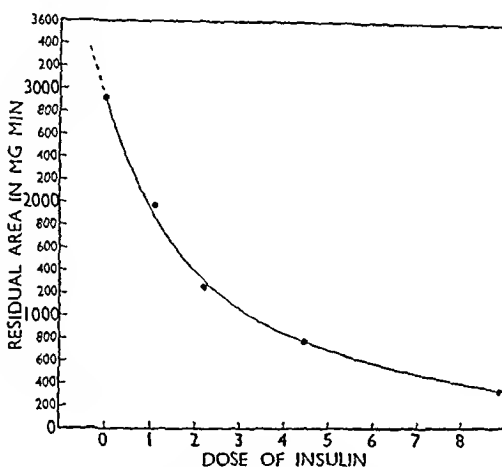


Fig. 8. Diagram showing the influence of the degree of alimentary hyperglycæmia upon the effect of standard increments of insulin in suppressing the hyperglycæmia. The application of these findings is given in the test. The curve is drawn from data published previously (22).

hyperglycæmia in facilitating insulin action. It will readily be seen that if the process of impairing insulin sensitivity is extended a point will soon be reached at which the glucose tolerance area becomes so large that it reveals the existence of diabetes and at this stage we shall have a diabetic with the characteristics of the insensitive type. The glucose tolerance area will be large, the corresponding insulin area will be approximately normal, and the I/G ratio will be small. It is on this basis of impaired sensitivity to insulin that we explain the insensitive type of diabetes. But the impairment of insulin sensitivity in this type of diabetic differs from that which occurs in healthy subjects in response to restriction of dietary carbohydrate in that the insulin-insensitive diabetic shows a gross impairment of insulin sensitivity even when he is receiving diets upon which he should be relatively insulin sensitive. This abnormally low sensitivity to insulin is practically constant and little influenced by dietary change. *It is therefore concluded that the*

insulin-insensitive diabetic reacts to insulin in the manner which would be expected if his disease were due, not to lack of insulin, but to impairment of insulin action.

It has long been known that the most characteristic action of insulin is that it increases the intake of sugar by the peripheral tissues (4) (12) (13) (30). If therefore the sensitive type of diabetes is due solely to lack of insulin, injection of insulin in these cases should still promote an increase in the rate of abstraction of sugar in the periphery, whilst if the insensitive type of diabetes be due to insensitivity to insulin the peripheral action of insulin in these cases might be expected to be impaired. In man and in intact animals change in the difference between the sugar content of the arterial or capillary blood and of the venous blood after injection of insulin has been

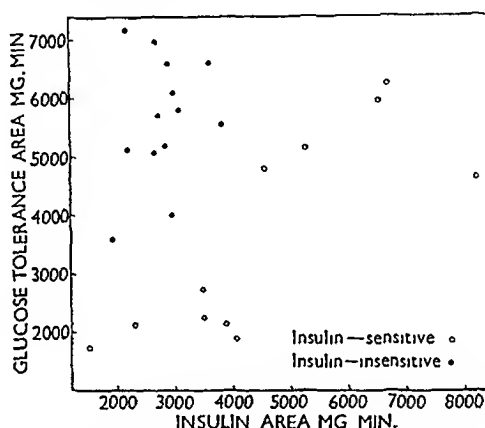


Fig. 9. Chart showing the correlation of the effect of a standard dose of insulin in suppressing alimentary hyperglycemia (measured by the insulin area in mg. min.) and the degree of alimentary hyperglycemia (measured by the glucose tolerance area in mg. min.) in insulin-sensitive diabetics (circles) and insulin-insensitive diabetics (discs). The composition of the diet was approximately the same for all patients. It will be seen that in the case of insulin-sensitive diabetics the insulin area varies greatly, whilst in the case of the insulin-insensitive diabetics the insulin area varies relatively little, despite the large variation in the glucose tolerance area in different patients.

used as a measure of the effect of insulin in the peripheral tissues. It has been shown by one of us (21) that in normal subjects and in sensitive diabetics the capillary-venous blood sugar difference is much greater after insulin and glucose than after glucose alone, whilst in insensitive diabetics this difference is little increased by administration of insulin along with the glucose. It was suggested that the two types of response to the insulin-glucose test shown by the diabetic patients could be largely explained as due to difference in the peripheral action of insulin. This work has been re-investigated by Griffiths (14) and his findings are qualitatively of the same nature as ours. He disagrees, however, in finding that the peripheral action of insulin is impaired in both types of diabetic, although the impairment is less marked

in the sensitive than in the insensitive type. This disagreement has led Griffiths to put forward a different explanation of the two types of diabetic response to the insulin glucose test. Whilst we suggest that the insensitive diabetic differs from both the normal subject and the sensitive diabetic in showing a diminished response to the peripheral action of insulin he suggests that the peripheral action of insulin is diminished in both types of diabetic and that the difference between the two types lies in an impairment of the ability of the liver to store sugar following injection of insulin. Griffith's explanation and ours are not mutually exclusive and both are compatible with the original suggestion that the sensitive type of diabetic is suffering from lack of insulin whilst the insensitive type is suffering from insusceptibility to insulin.

The early work of Frank, Nothmann and Wagner (13) (14) showing that insulin increased the arterio-venous blood sugar difference in depancreatized dogs, is relevant to the view that the sensitive type of diabetic is due to deficiency of insulin. These workers showed that in depancreatized dogs the arterio-venous blood sugar difference was diminished but that it could be increased as in the normal dog by injection of insulin. Similar results on young cases of human diabetics were reported by Lawrence (28).

Any enquiry into the nature of insulin-insensitivity must take into account the work initiated by Houssay and his school on the relationship of the anterior pituitary gland to carbohydrate metabolism. They have shown that hypophysectomy ameliorates pancreatic diabetes whilst hypophyseal extracts produce temporarily in normal animals a state similar to diabetes mellitus. The original paper on the differentiation of diabetics into two types by means of the insulin glucose test included the evidence that in insulin sensitive diabetics the peripheral action of insulin was impaired but at the time of its publication there was no evidence that extracts of the anterior hypophysis inhibited insulin action in the peripheral tissues. But shortly afterwards Marks (31) showed that decapitated eviscerated preparations, which had previously been treated with crude extracts of the anterior hypophysis, showed in response to injection of insulin a slower fall of blood sugar and smaller deposition of muscle glycogen than was shown by the control preparations. Later Himsworth and Scott (26) showed that the peripheral action of insulin was largely abolished in unanæsthetized rabbits from which the liver was excluded if they had previously been treated with an extract of the anterior hypophysis (Young's glycotropic factor (42, 44)). The difficulties in applying the work on the anterior pituitary gland to the explanation of insensitive diabetics was thus removed and in experiments shortly to be published (24) it will be shown that injection of certain extracts of the anterior pituitary gland into normal animals results in diabetic glucose tolerance curves and insulin glucose curves similar to those of insulin-insensitive diabetics. Soon after Marks's results became available de Wesselow and Griffiths (6) published results which promised that the rôle of the pituitary gland in certain cases of human diabetes, was to be

established. They claimed that the plasma from certain diabetics, who clinically were of the type giving the insensitive response to the insulin glucose test, produced a state of relative insensitivity in normal rabbits similar to that produced by injection of anterior pituitary extracts (40, 44). In conjunction with Dr. D. B. McNair Scott we undertook a repetition of this work but unfortunately we have been unable to confirm it.

Evidence that the pituitary gland is concerned in the mechanism of sensitivity to insulin in normal animals has been brought forward by Himsworth and McNair Scott (25). They showed that hypophysectomised rabbits, unlike normal rabbits, do not become insensitive to insulin when dietary carbohydrate is restricted but that such animals when given an extract of the anterior pituitary gland (Young's glycotropic factor) develop an insensitivity to insulin similar to that of normal rabbits when given a diet low in carbohydrate. The suggestion follows that low carbohydrate diets induce, whilst high carbohydrate diets restrain the secretion by the pituitary gland of a substance similar to the glycotropic factor. As we have previously mentioned in this present paper insensitive diabetics improve little if at all when the dietary carbohydrate is increased. If the insensitive type of diabetes is due to pathological over-secretion of a glycotropic like substance by the pituitary gland then it is not surprising that in these patients the physiological stimulus to restraint of this secretion, namely an increasing consumption of carbohydrate, is relatively ineffective.

The involvement of the anterior pituitary gland in the production of a diabetic syndrome has been established by Young (41, 43). He has shown in dogs that injection of a pituitary extract over a relatively short period of time will produce a state of diabetes which persists after the injections are stopped. During the period of injection these dogs give an insensitive response to the insulin-glucose test (45) and in this connection the two cases of Cushing's syndrome with associated diabetes mellitus which we have investigated are of interest. Both were definitely insulin-insensitive and in one of these cases the disease was so mild and, presumably so early, that it was only discovered by a routine glucose tolerance curve, and in the second case measures directed solely against the pituitary gland produced a marked diminution in the severity of the diabetes and a definite increase in sensitivity to insulin. But in the stage of permanent diabetes there is evidence that Young's dogs are deficient in insulin. Such animals show marked changes in the islets of Langerhans (36) and their pancreas contains no demonstrable amount of insulin (3). It appears that whilst the diabetes of these animals during the injection stage may be due to antagonization of endogenous insulin by pituitary factors during the stage of permanent diabetes their disease is due, at least in part, to insulin deficiency. And in this latter stage the dogs are sensitive to insulin (45).

To summarise, it can be said that there is considerable evidence that two types of diabetes can be differentiated on the basis of the speed with which they react to insulin. In one type, the insulin-sensitive type, insulin

comes into action rapidly, in the other, the insulin-insensitive type, insulin comes into action slowly. The experimental evidence is compatible with the suggestion that whilst the disease in the sensitive type of case is due to lack of insulin, in the insensitive type of case the disease is due, not to lack of insulin, but to impairment of insulin action. There is as yet no direct evidence as to the mechanism by which this insensitivity to insulin is produced although there is suggestive evidence that the anterior pituitary gland is concerned in the diabetes mellitus associated with hyperpituitarism and in the production by dietary variations of insulin insensitivity in normal animals.

SUMMARY.

1. Under standard conditions of diet and environment, diabetic patients react in one of two ways to the insulin-glucose test and by means of the test diabetics may be divided into two distinct types. In one type, the insulin-sensitive diabetic, the rate at which injected insulin comes into action is unimpaired; in the other type, the insulin-insensitive diabetic, the rate is retarded.

2. Insulin-sensitive diabetics react favourably to increase of dietary carbohydrate. Glycosuria does not increase, the fasting blood sugar level does not rise and the sugar tolerance and the sensitivity to insulin improve. In the case of the insulin-insensitive diabetic, on the other hand, increase of dietary carbohydrate causes increase in glycosuria, a tendency to higher fasting blood sugar levels, impairment of sugar tolerance and little or possibly no increase in sensitivity to insulin.

3. Corresponding to the two types of diabetics, differentiated by the insulin glucose test, two clinical groups of patients can be distinguished. The clinical distinction is not, however, precise as cases which clinically are of the type usually giving an insulin-sensitive response to the test may be found on testing to give an insulin-insensitive response, and *vice versa*. On the whole the insulin-sensitive diabetics tend to be younger, thin, to have a normal blood pressure and healthy arteries; in them the disease is sudden and severe at onset; they easily develop ketosis and react to a slight excess of insulin with a hypoglycaemic attack. The insulin-insensitive diabetics on the other hand tend to be older, obese, to have hypertension and to exhibit arteriosclerosis; in them the onset of the disease is insidious; they rarely develop ketosis and can tolerate over-dosage of insulin without showing symptoms of hypoglycaemia.

4. Two cases of Cushing's syndrome gave the insulin-insensitive type of response. In one case X-ray irradiation of the pituitary region was followed, not only by clinical improvement, but also by an improvement in the patient's sensitivity to insulin.

5. By combining the results of the blood sugar glucose tolerance and the insulin-glucose test data have been obtained which throw light on the

mechanism of the two types of diabetes. The data indicate that in the insulin-sensitive diabetic the disease is due primarily to lack of insulin, whilst in the insulin-insensitive diabetic the disease is primarily due, not to lack of insulin, but to impairment of insulin action. This insensitivity to insulin is of the nature of a retardation, rather than a neutralisation, of insulin action. In the insulin-sensitive diabetic the removal of sugar from the blood by the peripheral tissues under the influence of insulin is at a normal rate whilst in the insulin-insensitive diabetic it is impaired.

6. The relationship of the anterior pituitary gland to the phenomenon of insulin-insensitivity is discussed. Whilst there is no doubt that extracts of the anterior pituitary gland can produce a condition of insensitivity to insulin in normal animals there is as yet no evidence that insulin-insensitivity in human diabetics, save possibly in the diabetes associated with hyper-pituitarism, is due to excessive secretion of the anterior pituitary gland.

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AGE AND INSULIN SENSITIVITY.*

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IN a previous paper (2) a convenient method has been described for measuring the sensitivity to insulin of the human subject and in a subsequent paper (3) it has been shown that by means of this method diabetic patients can be differentiated into two types according to whether they are sensitive or insensitive to insulin. As in any particular case, the decision as to whether the case is sensitive or insensitive to insulin is made by comparison with the degree of insulin sensitivity in normal subjects it became necessary to determine the limits of variability of insulin sensitivity in healthy subjects and to decide if these limits were constant or varied under different conditions.

This method of determining sensitivity to insulin depends upon the principle that in a healthy subject the efficiency with which a constant dose of insulin suppresses alimentary hyperglycæmia depends upon the sensitivity of that subject to insulin. By measuring the blood glucose tolerance curve after ingestion of a standard dose of glucose, and the insulin-glucose curve after the intravenous injection of a standard dose of insulin and the ingestion of the same dose of glucose, data are obtained which permit the numerical expression of the degree of insulin sensitivity. The area below the glucose tolerance curve (the glucose tolerance area G) gives the degree of alimentary hyperglycæmia upon which the injected insulin acts; the area between the glucose tolerance curve and the insulin-glucose curve (the insulin area I) measures the effect of the injected insulin; the ratio of the insulin area to the glucose tolerance area (the I/G ratio) indicates the sensitivity of the subject to insulin (2). With increase of sensitivity the I/G ratio rises, with decrease of sensitivity it falls. This method is preferable to the direct method of estimating insulin sensitivity by measuring changes in the degree of depression of the fasting blood sugar after intravenous injection of insulin, because larger variations of blood sugar level being measured the results are less dependent upon the technical skill. It has already been shown by this method that in healthy young subjects insulin sensitivity varies with the composition of the diet (1) (2). In this paper evidence is presented that in healthy subjects insulin sensitivity varies with age.

* The expenses of part of this work were defrayed by a grant from the Lindley Diabetic Research Fund.

Methods.

Subjects. Thirteen subjects were investigated, two females and eleven males. Their ages varied from 18 to 64 years. Nine were healthy individuals admitted to hospital solely for the purpose of the test. Of the four other subjects, one had been admitted for investigation and nothing abnormal was found, one was receiving massage after a Pott's fracture experienced three months previously, one was convalescent after an electric burn and the last had recovered from a small operation in which fascia had been removed from the back muscles.

Preparation. All the subjects were in hospital and received a diet of 1750 calories, containing 175 g. of carbohydrate, for at least one week before the tests.

Tests. All tests were carried out under conditions as strict as those required for estimating the basal metabolic rate. Further details of the technique observed will be found in a previous paper (2). Two tests were

Table showing the change of glucose tolerance and insulin sensitivity with age in healthy subjects.

No.	Age.	Sex.	Glucose tolerance area (G) mg. min..	Insulin area (I) mg. min..	Residual area (R) (G-I) mg. min..	I/G
1	18	M	2900	2720	+180	0.94
2	19	M	2520	2360	+160	0.94
3	20	M	3168	3630	—462	1.14
4	21	M	2680	2640	+40	0.99
5	36	M	4190	4350	—160	1.04
6	45	F	3850	2040	+1810	0.53
7	45	M	{ 5750 6220	{ 4620 5070	{ +1130 +1150	{ 0.79 0.81
8	47	F	3860	3550	+310	0.92
9	58	M	4140	5180	—1040	1.25
10	59	M	4360	4210	+150	0.96
11	62	M	4340	2130	+2210	0.49
12	63	M	5490	3920	+1570	0.72
13	64	M	3640	2020	+1620	0.56

The tests were terminated at the end of one hour.

Duplicate experiments on the same patient are bracketted together.

carried out on each subject, a glucose tolerance test and an insulin-glucose test. The standard dose of glucose given by mouth in both tests was 30 g. per sq. m. of body surface; the standard dose of insulin injected intravenously in the insulin-glucose test was 5 units per sq. m. of body surface. Both tests were terminated after one hour.

Results.

The results of this investigation are given in the table. It will be seen that as age increases the glucose tolerance area tends to increase, that is, glucose tolerance diminishes. This finding is in agreement with the results of other investigators (4) (5). Parallel with this tendency to diminution of glucose tolerance there is a tendency to diminution of insulin sensitivity as

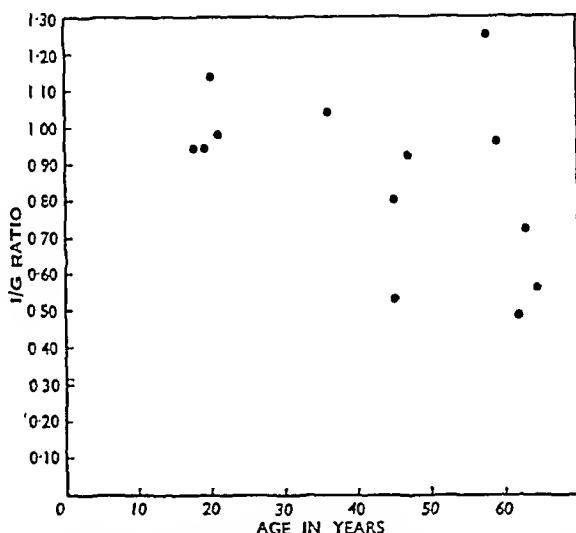


Fig. 1. Shows the relationship between the degree of insulin sensitivity, as measured by the I/G ratio, and the age of the subject.

shown by a diminishing I/G ratio. Thus the effect of age upon the glucose tolerance and the insulin sensitivity of healthy subjects provides another example of the correlation of these two properties. The same phenomenon can be seen in diabetic patients and it will suffice here to indicate this effect of age by giving the average I/G ratios. In healthy subjects under 40 years of age the I/G ratio, when taking a diet containing 175 g. of carbohydrate, averages 1.01; over 40, 0.78; in insulin-sensitive diabetics under 40 years 1.44, over 40 1.18; in insulin-insensitive diabetics under 40 years 0.56, over 40 years 0.49. Thus in both types of diabetic patients and in normal subjects sensitivity to insulin tends to decrease as age advances.

In the figure the ages of the normal subjects are charted against the I/G ratios. From this diagram it can be seen that in young healthy subjects

the I/G ratios tend to lie round the value 1.00 and that even in the older age groups individuals are found whose I/G ratios lie around this figure. But as age advances other subjects are found, with increasing frequency, whose I/G ratios lie at lower values. The fall in the average I/G ratio with age is to be attributed to the occurrence of such low I/G ratios in individual subjects rather than a general fall in the I/G ratios of all elderly subjects. These low I/G values are in some cases (subjects 6, 11 and 13) of the same degree as those found in insulin-insensitive diabetics and when compared with the I/G values in young healthy adults they indicate considerable impairment of insulin sensitivity. We regard the values of the I/G ratio in young healthy adults as indicative of normal insulin sensitivity and we consider the low values of the I/G ratio found in some elderly healthy subjects as indicating impaired sensitivity to insulin which is, however, of a degree still compatible with normal health.

Discussion.

It has been shown in a previous paper (3) that cases of diabetes can be divided into two distinct types according to whether they are sensitive or insensitive to insulin. The degree of sensitivity to insulin is indicated by the I/G ratio and in healthy young adults this averages 1.01. In thirteen insulin-insensitive diabetics the mean I/G ratio was 0.51, the standard deviation of the observations being 0.11. From the tables in the present paper it will be seen that in three normal subjects (subjects 6, 11 and 13) the I/G ratio lay within the distance of one standard deviation of 0.51 and that the value in five subjects of the series (subjects 6, 7, 11, 12 and 13) lay within three standard deviations of the mean. All five patients were over 40 years of age. It is thus clear that certain elderly healthy subjects may show degrees of insulin insensitivity of the same order as are shown by insulin-insensitive diabetics and the significance of this finding requires consideration.

Theoretically diabetes mellitus may result either from deficiency of insulin or from insensitivity to insulin the supply not being deficient. Evidence has previously been brought forward which suggests that in the insulin-insensitive diabetes the disease is due, not to lack of insulin, but to impaired sensitivity to insulin. If insulin sensitivity were to become increasingly diminished in a normal subject the effect of the pancreatic insulin in restraining alimentary hyperglycæmia would also progressively diminish and soon a stage would be reached at which the blood sugar tolerance curve would become definitely diabetic in form. The decreasing insulin sensitivity during this train of events would be indicated by a falling I/G ratio. At a stage just before the sugar tolerance curve became of the diabetic type the following state of affairs would exist. The tolerance for dietary carbohydrate would still be sufficiently high for the subject not to develop post-prandial glycosuria and the consequent symptoms of thirst and polyuria; the sugar tolerance curve would be higher than the average for

healthy subjects yet not sufficiently high to be characterised as diabetic in type; the insulin sensitivity would be markedly impaired as indicated by an I/G ratio which approached the low values found in insulin-insensitive diabetics. The suggestion that many apparently healthy subjects past middle life are potentially diabetic is not incompatible with clinical experience, for the incidence rate of diabetes increases after middle age and amongst middle aged and elderly diabetics the insulin-insensitive type of diabetes predominates.

It will be seen from the table that the values for the I/G ratio of the thirteen healthy subjects do not fall into separate groups but are distributed between the two extreme values. In diabetics on the other hand it has been shown (3) that the I/G values fall into two distinct groups. The question now arises as to why the I/G ratios in normal subjects grade from insulin-sensitive to insulin-insensitive values whilst the I/G ratios in diabetics are either at definitely insulin-sensitive or at insulin-insensitive values. These findings are easily explained if it is allowed that insensitivity to insulin can cause diabetes to develop. Diabetes mellitus due to insensitivity to insulin appears only in the presence of a high degree of insulin insensitivity. Therefore all insensitive diabetics have low values for the I/G ratio. I/G values intermediate between those of normal sensitivity and those found in the insulin insensitive diabetic indicate minor degrees of impairment of insulin sensitivity which are insufficient to produce diabetes mellitus. These intermediate values are found therefore in apparently healthy subjects.

SUMMARY.

1. The effect of age upon the sensitivity to insulin of thirteen healthy subjects, whose ages ranged from 18 years to 64 years, has been investigated.

2. The relatively high degree of sensitivity to insulin seen in healthy young adults is found in subjects of all ages but in the higher age groups subjects are frequently encountered in which insulin sensitivity is impaired to a greater or less degree. Such subjects are regarded as potential diabetics of the insulin-insensitive type and their impaired sensitivity to insulin as an abnormality which, however, is not sufficiently advanced to be incompatible with health.

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THE FIBRE DISSOCIATION PRODUCED BY COOLING HUMAN NERVES.

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IN a previous communication (1) I used the method of nerve cooling to differentiate the two sets of fibres which are involved in itching. Cooling has the advantage over other methods of nerve paralysis that it can be controlled delicately. It can be maintained at any stage or rapidly reversed. The type of dissociation produced differs from that of cocaine and asphyxia. It would therefore be of considerable interest to correlate the present results with those obtained oscillographically from a mammalian nerve after graded cooling. Such observations do not appear to be at present available.

Method.

The apparatus consists essentially of a metal cooling element placed in close contact with the skin overlying a nerve. This element is made from lead tubing of a thickness that can be bent easily by hand. The width of the tubing depends on the size of the nerve to be cooled. For the ulnar nerve, a piece of lead tubing 1 cm. bore and 18 cm. long is bent into the shape of the ulnar groove when the elbow is flexed to a right angle. It is held by embedding the elbow on the top of a sand bag with the tube in place. For smaller cutaneous nerves, a lead tube (0.7 cm. bore) is partially flattened and held in place by two rubber bands and wooden cross pieces (Fig. 2). In this case the position of the nerve is first outlined on the skin by means of a faradic stimulus. The element can then be placed accurately over the nerve. A rotary pump circulates water through the cooling element. It is supplied by two reservoirs, one containing water at 39°C, and the other water or freezing mixture (ice and salt) at the required temperature. The temperature of that part of the cooling element which lies over the nerve is most conveniently measured thermoelectrically. For this purpose a thermal junction is soldered into the lead tubing at the point where it lies on the skin. Similar junctions applied to the skin have also been employed

* Work undertaken on behalf of the Medical Research Council.

to measure temperature changes in the territory supplied by the cooled nerve.

A simple technique has been employed for testing sensory changes. Cold and warmth are tested by the momentary application of a copper block which has been immersed in water at 12° and 40° respectively. A fine camel hair brush has been used for testing touch and tickle sensations. For pain sensation, a fine needle mounted on the end of a Von Frey hair (2.0 g.) has been used.

The results to be presented have each been obtained on five subjects accustomed to such observations.

Results.

Temperature of cooled nerve. Subcutaneous thermal junctions can be placed in close vicinity to the nerve. These indicate that when equilibrium has been attained (5 min.) the nerve has a temperature from 5° to 10° higher than the element (cooling range—5° to 15°).^{*} Since the temperature gradient between the nerve and element will depend largely on the amount of insulating subcutaneous fat present, it is clear that no absolute correlation between the temperature required to produce a certain degree of paralysis can be expected in different subjects, or in different nerves of the same subject. In certain subjects possessing an unusual amount of subcutaneous fat it has been impossible completely to paralyse all fibres with safe degrees of cooling.

Order of paralysis. The nerve may be subjected to sudden severe cooling (perfusion of salt water at —5°) or the cooling may be rendered more gradual by using higher temperatures. The order of fibre paralysis is the same in either case, but rapid cooling produces certain additional changes which will be described separately. The following description applies to the ulnar nerve cooled in 5° stages, each stage lasting about 6 min.. The ulnar nerve is chosen because it supplies a relatively large area suitable for sensory testing, and it contains motor and vasomotor, in addition to sensory fibres.

No sensory changes can usually be detected until the element temperature has fallen to 15°. It is then noticed that the tickle sensation elicited by lightly stroking with a camel hair brush is diminished over the hypothenar eminence. At this time the sensation of cold becomes impaired over the same area. With further cooling these changes become intensified and spread in a peripheral direction into the little and ring fingers. It has frequently been observed that shortly after the sensation of cold has been abolished the application of the cold copper block results in a sensation of pure warmth, free from sting or burning. At the same time the whole ulnar area gives a slight spontaneous sensation of warmth although actual measurements show no change in skin temperature. At this stage, immersion

^{*} When using the low temperatures the skin does not freeze at first owing to supercooling. At the first sign of freezing, however, the experiment must be stopped as otherwise permanent damage to the nerve may result.

of the little finger in water at 10° produces no feeling of cold, showing that the paralysis of this sensation is complete.

It is more difficult to determine the exact point when tickle is lost; but it seems to correspond approximately with the loss of cold sensation. Coincident with the disappearance of tickle, the "itchy" skin surrounding an itching lesion is abolished, as previously reported (1). The skin feels very numb at this stage of cooling. This numbness is first detected on the

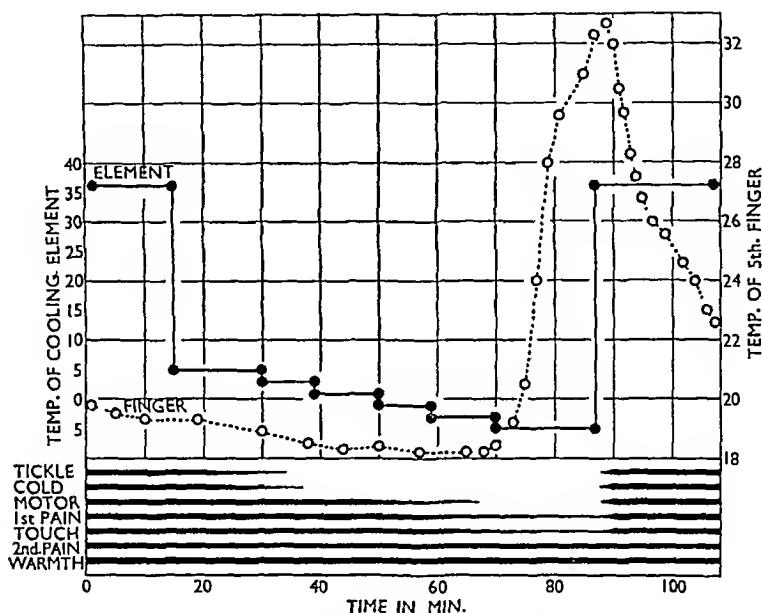


Fig. 1. R.G.B. Relating the paralysis of vasomotor, motor, and sensory fibres when the ulnar nerve is cooled by a metal element at the elbow. The vasomotor paralysis is shown by recording the skin temperature at the tip of the corresponding 5th finger. The times at which the various sensations disappear, and reappear after warming are indicated below.

hypothenar eminence shortly after tickle becomes impaired. It spreads likewise in a peripheral direction and becomes intensified. This numb skin gives a weakened 1st pain response (5), and this is associated with an intensification of the 2nd pain response. The latter becomes very long sustained and itchy in character.

With a little further cooling, the power in the muscles supplied by the ulnar becomes rapidly weakened, and there is a feeling of fatigue during voluntary contraction. Complete muscular paralysis is followed almost immediately by a feeling of warmth in the ulnar area, the skin temperature rising rapidly, as will be seen in Fig. 1. Shortly after this vasodilatation the touch sensation is completely lost, and 1st pain also disappears.

The remaining sensations, namely, warmth, 2nd pain, and "spontaneous itching" (1) require for their paralysis, a considerably greater degree of

cooling; the temperature of the cooling element has usually to be reduced to -5° , and even at this temperature they remain resistant in many subjects. When paralysis occurs, 2nd pain and "spontaneous itching" disappear before warmth, which is the last sensation to be affected.

It will thus be seen that cooling produces a dissociation of function, and that the various nerve fibres may be grouped according to their susceptibility to cold. The most susceptible are those subserving the sensations of cold, tickle, and "itchy skin." The most resistant are those subserving 2nd pain, "spontaneous itching," and warmth; while motor, vasomotor, 1st pain, and touch fibres form an intermediate group.

When the nerve is rewarmed to 37° a rapid recovery of function ensues, and is complete within 1 to 2 minutes. By rewarming more gradually it can be shown that the order of recovery of function is the exact reverse of that produced by cooling.

Paralysis of sweat fibres. Since palmar sweating is not easily induced experimentally the forearm nerves have been used instead of the ulnar to demonstrate the cold paralysis of sweat fibres. The cooling element is applied, and the nerve territory prepared with starch and iodine (Minor's method (7)). Copious sweating is now induced by immersing the legs of the subject in a hot bath. If the nerve is cooled sufficiently to abolish the touch sensation, no sweating occurs within its territory although the rest of the arm sweats profusely. On slightly warming the nerve so that the cold sensation begins to recover, sweat appears immediately across the whole nerve area (Fig. 2). Thus sweat fibres appear to be paralysed at approximately the same stage as the vasomotor fibres of the ulnar nerve.

Effect of rapid nerve cooling. The most conspicuous effect of rapid nerve cooling is the intense aching pain felt locally under the cooling element and within the nerve's territory. It continues until fibre paralysis has advanced to the stage of considerable numbness, and then it rapidly disappears. Its occurrence is associated with the development of marked hyperalgesia in the nerve area. The latter may continue after the spontaneous pain has disappeared, but is finally abolished in conjunction with touch and numbness. The presence of hyperalgesia within the ulnar area is associated with finger tip tenderness similar to that reported by Lewis and Pochin (6) as occurring during asphyxial paralysis of nerves. The pain clearly results from rapid refrigeration acting as a temporary nerve stimulus. The nature of hyperalgesia and the associated finger tip tenderness is little understood, but it seems probable that they are also to be interpreted as resulting from stimulation of pain nerves under the cooling element. In other respects the dissociation produced by rapid nerve cooling is identical with that resulting from the more gradual process.

Comparison of the dissociation produced by cooling different nerves. Observations have been made on the type of dissociation produced by cooling the following nerves; the ulnar, various forearm cutaneous nerves, the digital nerves and the external popliteal nerve. These nerves only present

differences in regard to their vasomotor fibres, which will therefore be considered separately.

The vasomotor paralysis. In the ulnar nerve vasomotor paralysis has already been noted to follow shortly after that of the motor fibres. All the vasomotor fibres appear to be about equally susceptible to cold. Thus their paralysis is very rapid and complete (Fig. 1). Moreover a slight decrease in the cooling temperature results in a complete return of constrictor tone. When the vasoconstrictors have been paralysed for 3-5 min. a slight flush is often visible over the whole ulnar area. It is blotchy in character, and disappears slowly after recovery of the cooled nerve.

The vasomotor response of the ulnar nerve to cooling contrasts with that obtained when a forearm nerve is cooled. In the latter case there is no rise of temperature, or flushing in the nerve territory, even though the cooling is taken to the stage of complete sensory paralysis. When at this stage the subject's body is warmed by immersing the legs in a hot water bath, a generalised vasodilatation is produced, but this does not affect the area supplied by the cooled nerve. The latter area warms and becomes flushed when the cold block is released sufficiently to allow the touch sensation to recover. These observations resemble closely those obtained by Grant and Holling (3) using procaine block of cutaneous nerves. They explained the differences in the behaviour of the forearm and the ulnar nerves by supposing that the former contained predominantly vasodilator, and the latter predominantly vasoconstrictor fibres.

Comment.

The dissociation is not due to asphyxia. An objection might be raised to the present experiments that the dissociation results in part from the effect of pressure on the cooled nerve. It is known that pressure applied to a nerve for 15 min. asphyxiates it sufficiently to produce considerable numbness within its distribution (4). Since the cooling experiments may last over an hour, there would be ample time for the development of asphyxial effects, given sufficient pressure on the nerve. The experiments of Waller (8), who produced an ulnar paralysis by pressing the elbow on a block of ice, are open to the same criticism. In the present experiments the complete recovery of function, upon rewarming the element *in situ* shows that asphyxia due to pressure is negligible. There is, however, the further possibility that cold itself asphyxiates the nerve by reducing its blood supply. On rewarming, the circulation to the nerve would be restored, so that a complete recovery would not distinguish between asphyxial and cooling effects. The two can, however, be distinguished if the limb circulation is arrested by a pneumatic cuff on the arm, before rewarming is commenced. It is then found that functional recovery is complete on rewarming although the return of blood supply to the nerve has been prevented.

Mechanism of fibre paralysis. The dissociation of nerve functions produced by cooling might be due to the temperature gradient across a nerve

in which the fibres are grouped according to function. Such an explanation might be possible in larger nerves such as the ulnar, but in the smaller cutaneous nerves there could be no significant fall of temperature across the width of the nerve.

A more likely hypothesis is that nerve fibres subserving different functions are unequally susceptible to cold. It is already known that these fibres exhibit a graded susceptibility to asphyxia, and to cocaine, and it seems probable that cold produces a dissociation of nerve functions in a similar manner. More recently it has been suggested that this varying susceptibility is to be correlated with fibre diameter, large fibres being most susceptible to asphyxia and least susceptible to cocaine. It is therefore relevant to inquire whether fibre diameter may be the determining factor in susceptibility to cold.

For this purpose the order of fibre paralysis produced by cold, cocaine, and asphyxia are displayed schematically below. The data for cocaine and asphyxia have been taken from Gasser (2), and Lewis and Pochin (6), respectively.

<i>Cold.</i>	<i>Asphyxia.</i>	<i>Cocaine.</i>
Cold	Touch	Vasomotor
Motor	Motor	Cold
Vasomotor	Cold	Warmth
1st pain	1st pain	2nd pain
Touch	Warmth	1st pain
2nd pain	2nd pain	Motor
Warmth	Vasomotor	Touch

It will be seen that the cold dissociation differs considerably from both those of asphyxia and cocaine. The position of the vasomotor fibres, which are known to be small, between those of motor and touch, which consist of predominantly large fibres, makes it clear that fibre size is not the determining factor in the cooling dissociation.

SUMMARY.

A simple method of paralysing human superficial nerves by cooling is described. The degree of paralysis can be accurately regulated, enabling the resulting dissociation to be studied in detail. The order of fibre paralysis differs from that produced by both asphyxia and cocaine.

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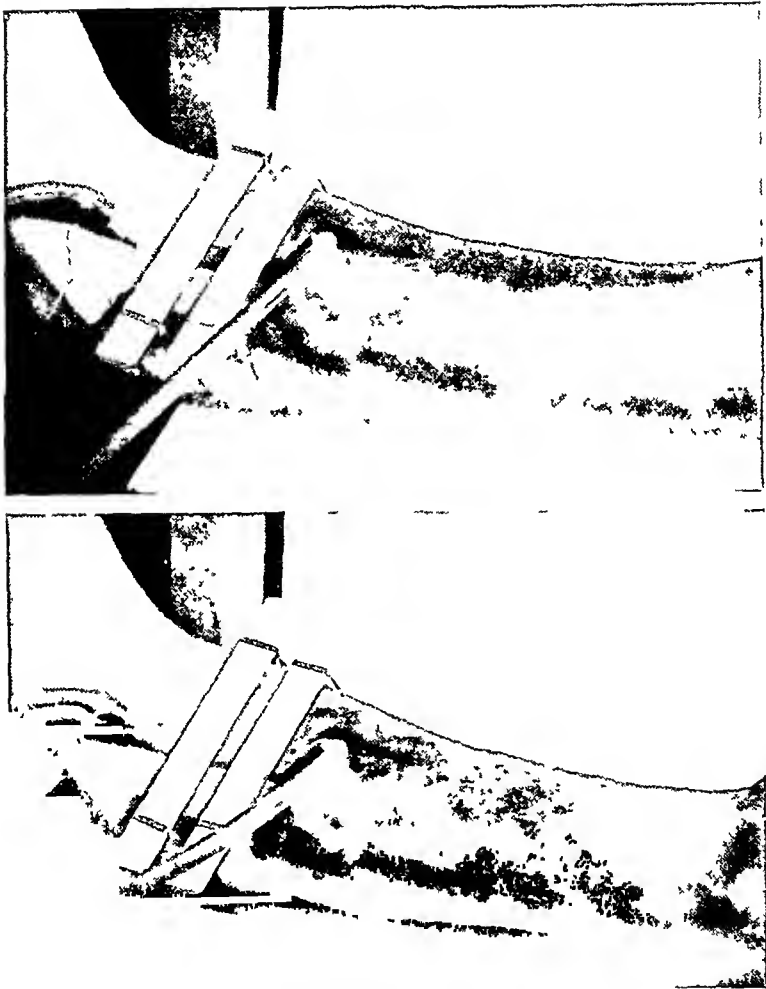


Fig. 2 R G B Showing the paralysis of sweat fibres by cooling. Sweating is displayed as a blackened area by the starch iodine method. In the upper figure sweating produced by warming the body is prevented in the territory of a forearm nerve cooled to 0° by the metal element. In the lower figure on warming the element to 37° sweating occurs over the whole territory.

A RAPID METHOD OF DETERMINING LUNG CAPACITY.

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Introduction.

THERE is at present an obvious need in studies of respiratory disease for a method by which the volume of air in the lungs may be determined with ease and accuracy. Existing methods are almost invariably complex with the exception of that devised by Christie (1). This method, however, has been shown to be liable to considerable systematic error and, even when this is corrected by the method suggested by Herrald and McMichael (3), the standard error of the method still remains ± 0.18 litre for single determinations. Determination of the lung volume by this method occupies two skilled workers for an hour, one being responsible for making the respiratory record while the second carries out the gas analyses in duplicate.

In the modified Christie method, use was made of a katharometer to follow the changes in oxygen percentage in the system. It was pointed out that this apparatus was insufficiently accurate to be a substitute for ordinary gas analysis necessary at the end of the mixing period. The katharometer, however, is extremely accurate in the analysis of hydrogen in air, and it seemed that by the use of hydrogen as the diluent gas, the katharometer might be a substitute for the more difficult methods of gas analysis otherwise required. This technique has been found to be entirely successful. The total time required for duplicate determinations of the lung volume and its sub-divisions is not more than half an hour for a single observer.

Principle of the method.

The principle of the katharometer is shown diagrammatically in Fig. 1. When two equal resistances (r_1 and r_2) enclosed in small metal cells and connected to the arms of a balanced Wheatstone bridge, are exposed to

* I am deeply indebted to Dr. L. E. Bayliss for much assistance and advice in developing this technique. The work was carried out while holding the Johnston and Lawrence Research Fellowship of the Royal Society and expenses were defrayed by a grant from the Lawrence fund of the Royal Society.

gases of the same thermal conductivity the resistance of each will be the same and no current will flow through the galvanometer. If, however, one of the cells contains a gas of higher thermal conductivity, cooling takes place and the resistance will increase, leading to unequal currents in the two arms of the Wheatstone bridge, and a current will flow through the galvanometer. Hydrogen has a much higher thermal conductivity than air, and thus when a mixture of air and hydrogen is used in one of the cells, and air alone in the

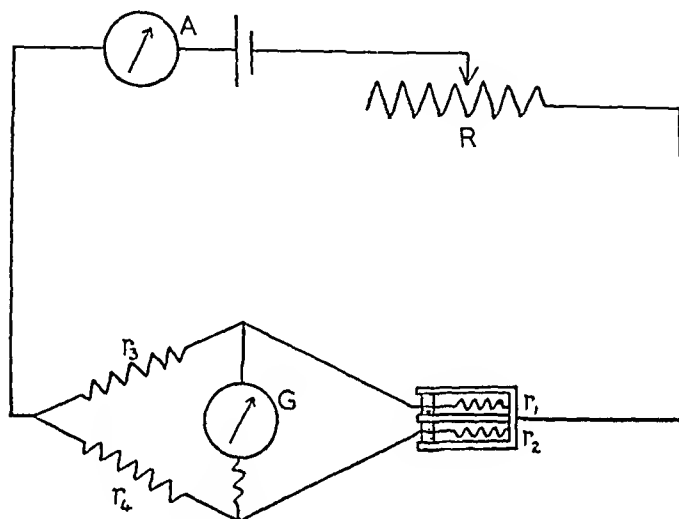


Fig. 1. The Principle of the Katharometer. For description see text.

other, deflections of the galvanometer needle can be produced which vary with the sensitivity of the instrument. The apparatus at present in use consists of a standard katharometer supplied by the Cambridge Instrument Company, with a galvanometer of 20 ohms resistance and 4.8 millivolts full scale deflection. With a katharometer current of 110 milliamperes, 12.5% of hydrogen in air gives a full scale deflection. By using air-hydrogen mixtures of known composition the galvanometer deflections may be calibrated at different katharometer currents, for example, from 100 to 130 milliamperes, so that the hydrogen percentages can be read from several curves (Fig. 2).

The actual apparatus used is the same as that described previously (3). The katharometer chamber is inserted in a Knipping spirometer circuit just beyond the soda lime scrubber. When the gas mixture in the spirometer is circulated by means of the air pump it diffuses into the katharometer chamber and, if it contains hydrogen, a corresponding deflection of the galvanometer needle is produced. The modifications of the ordinary Knipping spirometer which are required for this work have been fully described. For use with hydrogen the only addition which has to be made

is that of a gas measuring cylinder, from which a known volume (for example 500 c.c.) of hydrogen may be added to the spirometer.

The hydrogen used is obtained from the British Oxygen Company, which supplies cylinders of the gas guaranteed to be at least 99.9% pure, the only impurity being oxygen.

Technique.

The determination is carried out in two stages. (1) An ordinary spirometer tracing is taken with the apparatus containing an oxygen-air mixture. During this recording, tracings are made of the vital capacity as well as of the complemental and reserve airs recorded separately. These tracings are always taken first as this has the advantage of accustoming the patient to breathing in the apparatus so that regular tracings are obtained easily during the second stage of the procedure. (2) The apparatus is washed out with atmospheric air and the current to the katharometer circuit is switched on. The galvanometer reading must return to the zero position as an index that no hydrogen has been left in the apparatus from previous estimations. The volume of the spirometer dead space in the writer's apparatus is 2.6 litres. Using the scale at the side of the apparatus, the spirometer bell is set at 0.9 litre above the zero level, so that the whole apparatus contains 3.5 litres of atmospheric air. The spirometer system is closed, and 500 c.c. of hydrogen are added from the measuring cylinder. The gas mixture in the spirometer is then circulated by means of the pump until complete mixing has taken place including diffusion into the katharometer chamber. At a given katharometer current, measured by the milliammeter, the deflection produced by (500/4000) 12.5% H_2 is known. The calibration should be checked in each experiment by making sure that the hydrogen mixture in the spirometer gives the expected galvanometer reading.

The patient is now switched into the spirometer circuit and oxygen is added from a cylinder with a calibrated flow meter at a uniform rate to balance approximately the rate of oxygen consumption. As the spirometer tracing proceeds the galvanometer needle shifts towards a new position, at first rapidly and then more slowly until finally a stationary position is reached. At the end of this time, which is usually from 4 to 6 minutes, complete equilibrium is reached throughout the lung-spirometer system and the patient may be disconnected. If, however, the respiratory tracing shows any irregularity, which would make it difficult to assess the position of the resting respiratory level, it is advisable to record the reserve air shortly before finishing the spirometer tracing.

From the galvanometer deflection the percentage of hydrogen in the system may be estimated from two or three readings taken on a series of calibration curves at different katharometer currents (Fig. 2). Using this technique it is found that the hydrogen percentage may easily be estimated with an accuracy of $\pm 0.02\%$.

Calculation.

Let x litres = volume of air in the lungs at the end of a normal expiration (functional residual air). Suppose the final percentage of hydrogen in the spirometer circuit to be a . Suppose also the volume of the

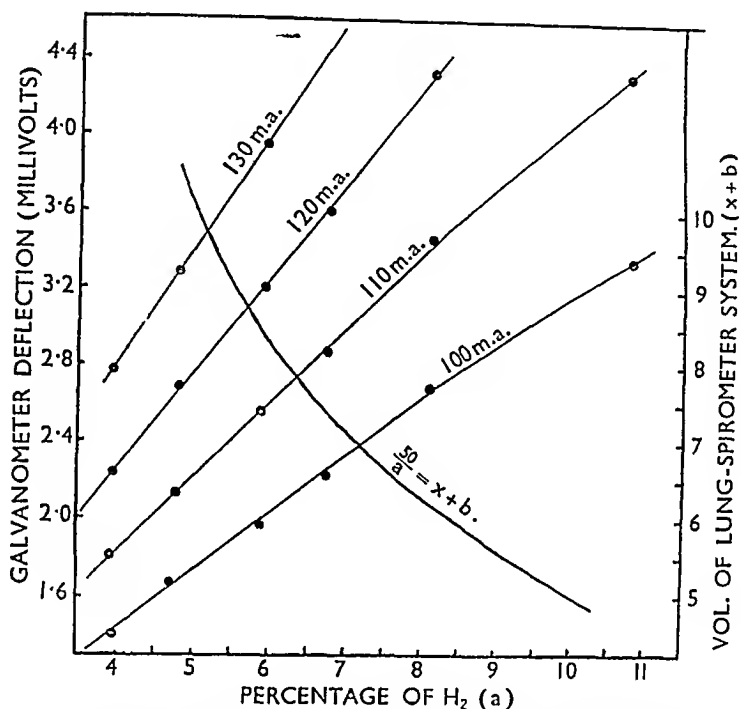


Fig. 2. Calibration curves giving the percentage of hydrogen from the galvanometer reading at different currents. The volume of the lung spirometer system may be read from the graph of $50/a = x + b$.

spirometer circuit at the end of a normal expiration to be b (this value can readily be observed directly at the end of the second tracing). Then, since the volume of hydrogen in the system is 0.5 litre,

$$\frac{0.5}{x + b} \times 100 = a.$$

$$\text{Whence } \frac{50}{a} = x + b.$$

a and b being known quantities, x may readily be calculated.

In practice a graph of the equation $\frac{50}{a} = x + b$ may be made for values of a ranging from 5 to 10 per cent. (Fig. 2). From this graph $x + b$ can be read off, and x obtained by subtracting b .

Corrections. The figure calculated from the above equation will obviously require various corrections in accordance with the gas laws. In addition to this there are one or two errors which may be regarded as constant.

(a) Constant errors :—

1. Dead space of the mouthpiece of the spirometer.

When the subject is switched into the spirometer circuit there is in each instrument a certain volume of air in the metal mouthpiece, which can readily be estimated and which should be subtracted from the calculated value of the volume of air in the lungs. In the apparatus used this dead space was 60 c.c..

2. Dead space of the respiratory passages.

The calculated volume of air in the lungs includes the ordinary dead space air. Since this is liable to some variation in different individuals it is better to include this in the lung volume. Alternatively, an average value, for example 150 c.c. may be subtracted, or, if greater accuracy is desired, a separate estimation of the dead space air may be made.

3. The solution of hydrogen in blood and body fluids.

The solubility co-efficient of hydrogen in blood at 37°C. may be taken as 0.015. If we assume that the blood becomes fully saturated with hydrogen at the partial pressure present in the lungs, it can be calculated that the volume of hydrogen disappearing in 5 litres of blood will lie between 4.1 and 5.47 c.c. for functional residual air values between 4 and 2 litres. The error created by the disappearance of this volume of hydrogen from the lung spirometer system is corrected by the subtraction of 70 c.c. from the value calculated by the above equation.

4. Increase in concentration of inert gases in the lungs.

The above calculation assumes that the inert gaseous composition (H_2 and N_2) of the air in the lungs is identical with that in the external spirometer circuit. This is only true if the respiratory quotient is unity. With a respiratory quotient about 0.8 the actual volume of the functional residual air is usually about 30 c.c. less than that calculated. Although this value will change with variations in respiratory quotient and the lung volume, the assumption of this figure produces only a negligible error.

It has been the practice in this work to neglect the dead space of the respiratory passages, and to subtract constant values, namely, 60 c.c., 70 c.c. and 30 c.c. for the instrumental dead space, hydrogen solution and pulmonary gaseous concentration respectively.

(b) Variable errors :—

Errors of varying magnitude result from changing temperature conditions. The calculation made in the above equation assumes that the air in the lungs is at the same temperature as the air in the spirometer. Actually the lung air is at 37°C. and corresponding corrections must be made.

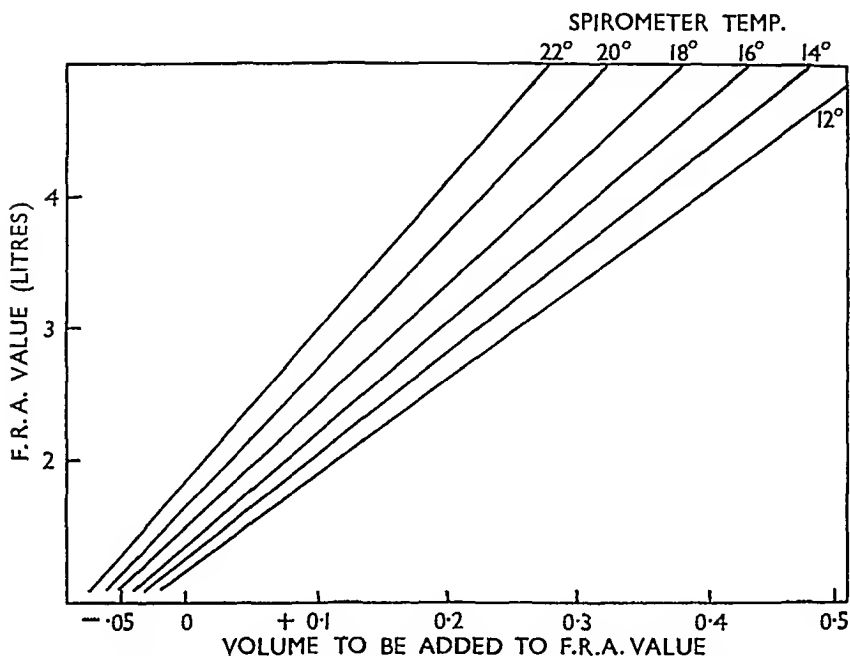


Fig. 3. Corrections to be made to the functional residual air values at different spirometer temperatures.

1. Simple temperature correction.

The actual volume of air in the lungs will be :

$$x \times \frac{273 + 37}{273 + \text{temperature of air in spirometer.}}$$

For example, if the spirometer temperature is 20°C. at the end of the observation x must be multiplied by 310/293 to obtain a true value.

2. Vapour pressure correction.

The air in the lungs is nearly saturated with vapour pressure at body temperature. The actual figure calculated by Christie and Loomis (2) may be taken as 45 mm. Hg.. At 20°C. the vapour pressure in the spirometer will be 17.5 mm. Hg.. The correction to be made therefore will be to multiply x by 760/732.5.

Combining these two correction factors at 20°C. x has to be multiplied by 1.097.

For convenience the volume to be added at varying functional residual air values may be calculated from the correction factors at different temperatures. The constant errors given under (a) above may be subtracted and a graph made from which the corrections may be read off at the time of each determination (*See Fig. 3*).

The accuracy of the method.

In 25 paired determinations of functional residual air the average deviation from the mean value was 0.068 litre, and the standard error of a single determination was calculated to be 0.09 litre. The method is thus much more accurate than the previous method of Herrald and McMichael in which the standard error was 0.18 litre.

SUMMARY.

A method is described by which the volume of air in the lungs may be determined easily and rapidly.

A measured volume of hydrogen is introduced into a Knipping spirometer from which the subject rebreathes until complete mixture of the gases is achieved. The final percentage of hydrogen is estimated from a galvanometer reading, the galvanometer being connected to a katharometer in the spirometer circuit. From this figure the total lung spirometer volume may be calculated, and the subtraction of the spirometer volume gives the volume of air in the lungs.

Details of various corrections are given, and the standard error of a single determination is \pm 0.09 litre.

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POSTURAL CHANGES IN THE LUNG VOLUME.

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It is now a matter of common knowledge that when the recumbent position is assumed the vital capacity tends to diminish (5). The reserve air decreases while the complemental air undergoes a distinct but scarcely compensating increase (1) (9) (18).

With regard to changes in total lung volume, there is still some doubt as to their exact nature. The earlier writers, Bohr (4) and Plesch (15), found that the residual air increases in the recumbent position, thus compensating to some extent for the diminution of vital capacity. More recent observers (1) (7) (9), however, have found either little change or a slight diminution in the residual air value in recumbency. Modern work thus supports the occurrence of a diminution of the total lung volume in the lying position. There is still, however, a difference of opinion as to how this diminution of the total volume of the fully expanded lung is produced. Hamilton and Morgan (7) made various measurements of the chest size by external and radiographic mensuration in the erect and prone positions. They concluded that as the chest capacity was slightly larger when the subject lay down, the diminution of total lung volume which occurred was due to increased pulmonary engorgement in this position. Some support for this contention was adduced from the observation that damming back blood in the limbs by pressure cuffs could also increase the vital capacity. Hurtado and Fray (9), however, found, in the horizontal position, a diminution in the radiological area of the fully expanded chest, this being due to a slightly higher position of the diaphragm. Both these groups of observers used the prone position for taking the chest radiographs, which might interfere with the achievement of full inspiration. In view of the discrepancies it was decided to repeat these investigations using slightly different technical procedures.

Material and methods.

The lung volume determinations were carried out by the hydrogen method previously described (13). Untrained subjects were taken at random

We are indebted to Dr. R. McWhirter for helpful criticism on the radiographic methods employed. Dr. R. Barry assisted us in many experiments. The expenses have been defrayed by a grant from the Lawrence fund of the Royal Society.

among technicians, medical men, and hospital patients suffering from diseases not known to affect the respiratory system. In certain selected subjects radiograms were taken in both erect and recumbent positions in full inspiration. In taking the radiographs the films were placed posteriorly and the tube, which was at 1.5 metres distance, was centred over the junction of the third ribs with the sternum. On achieving full inspiration the subjects snapped their fingers as a signal for the exposure to be made. Special precautions were taken in the radiographic procedures which are described below. The age distribution of the subjects was as follows:—

Age.	Number.
16-19 ...	7
20-29 ...	12
30-39 ...	3
40-54 ...	3

Results.

The results obtained in 25 subjects in the sitting and supine positions are shown in Table I, and the more significant means and distributions of

TABLE I.

Lung volume sub-divisions in the sitting and supine positions in 25 subjects.

Sub. No.	Ht. in ins.	SITTING.				SUPINE.				De- crease in T.L.V.	De- crease in F.R.A.	De- crease in R.A.		
		T.L.V.	R.A. abs.	%	F.R.A. abs.	%	T.L.V.	R.A. abs.	%				F.R.A. abs.	%
1	68	5.55	1.95	35	3.35	60	5.04	1.34	27	2.24	44	0.51	1.11	.61
2	69½	5.53	1.28	23	2.93	53	5.32	1.27	24	2.17	41	0.21	0.76	.01
3	66	6.32	1.27	20	2.92	46	5.92	1.07	18	2.17	37	0.40	0.75	.20
4	70½	5.95	1.05	18	2.50	42	5.61	1.31	23	1.71	30	0.34	0.79	+ .26
5	70	5.63	1.38	24	2.83	50	5.56	1.56	28	2.41	43	0.07	0.42	+ .18
6	65	5.40	1.75	32	2.55	47	5.00	1.25	25	1.55	31	0.40	1.00	.50
7	63	5.91	2.31	39	4.06	69	5.63	2.29	41	3.43	61	0.28	0.63	.02
8	72	7.84	1.99	25	4.34	55	7.28	2.08	29	3.58	49	0.56	0.76	+ .05
9	68½	6.58	2.43	37	3.98	60	6.13	1.98	32	2.93	48	0.45	1.05	.45
10	69	7.42	2.38	32	4.28	58	7.36	2.41	33	3.51	48	0.06	0.77	+ .03
11	68½	5.07	1.22	24	2.72	54	5.06	1.21	24	2.06	41	0.01	0.66	.01
12	62	4.68	1.41	30	2.65	57	4.52	1.36	30	2.06	46	0.16	0.59	.05
13	70	7.03	2.46	35	4.53	64	6.59	2.10	32	3.55	54	0.44	0.98	.36
14	65	5.32	1.97	37	3.22	60	4.77	1.57	33	2.62	55	0.55	0.60	.40
15	68½	6.22	1.37	22	2.52	40	5.96	1.46	24	2.16	36	0.26	0.36	+ .09
16	68	5.53	1.43	26	2.63	47	5.11	1.26	25	1.96	38	0.42	0.67	.17
17	71½	7.95	2.12	27	4.12	52	7.39	1.79	24	3.04	41	0.46	1.08	.33
18	67	5.78	1.33	23	2.93	51	5.59	1.24	22	2.29	41	0.19	0.64	.09
19	76½	7.61	2.43	32	4.66	61	7.36	2.51	34	3.86	52	0.25	0.80	+ .08
20	65	4.83	1.48	31	2.83	59	4.46	1.06	24	1.91	43	0.37	0.92	.42
21	68½	5.23	1.83	35	3.03	58	5.06	1.71	34	2.61	52	0.17	0.42	.12
22	75½	8.35	2.05	25	4.85	58	8.09	2.09	26	3.39	42	0.26	1.46	+ .04
23	68	5.93	1.28	22	2.68	45	5.63	1.28	23	2.38	42	0.30	0.30	± .00
24	73	7.33	1.78	24	4.03	55	6.62	1.42	21	2.82	43	0.71	1.21	.36
25	63½	4.38	1.38	31	2.43	55	3.95	1.03	26	1.65	42	0.43	0.78	.35

The standard error of each absolute value is ± 0.09 litre.

The standard error of each difference is ± 0.13 litre.

the results are summarised in Table II. It is seen that the relative values of residual air and functional residual air do not differ significantly in mean or in range from those found by Aslett, Hart and McMichael (2) in the sitting posture, but are somewhat higher than the figures obtained by Hurtado and Boller (10) in the supine position. The average postural change in lung

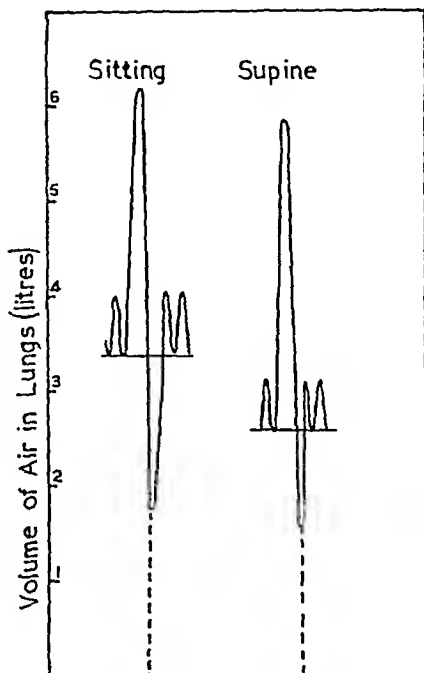


Fig. 1. Diagrammatic representation of the postural changes in the respiratory tracing based on the average results in 25 normal subjects. Upstroke = inspiration. Dotted line = residual air.

volume is shown diagrammatically in Fig. 1. It is seen that the more significant changes in the recumbent position are :—

1. A pronounced decrease in functional residual air (780 c.c.).
2. A decrease in total lung volume (340 c.c.).
3. A slight decrease in residual air (150 c.c.).
4. A decrease in vital capacity (190 c.c.).

The alteration in respiratory level.

When the recumbent position is adopted the functional residual air decreases by 780 c.c. in the average. When respiratory tracings are made

TABLE II.

Changes in lung volume with posture (in litres).

	<i>Sitting.</i>		Range.	<i>Lying.</i>		Range.
	mean	s. d.		mean	s. d.	
<i>Absolute values.</i>						
Total lung volume	6.14	1.03	4.38-8.35	5.80	1.03	3.95-8.09
Residual air	1.73	0.47	1.05-2.46	1.58	0.43	1.03-2.51
Functional residual air	3.34	0.59	2.43-4.85	2.56	0.63	1.55-3.86
<i>Relative values.</i>						
Residual air % T.L.V.	28.4	5.9	18-39	27.3	5.5	18-41
Functional residual air % T.L.V.	54.3	6.9	40-69	44.0	7.1	30-61
<i>Reduction in supine position.</i>						
Total lung volume	0.34	0.17	0.01-0.71			
Functional residual air	0.78	0.25	0.30-1.46			
Residual air	0.15	0.23	0.61-- + 0.26			

with the subject on a tilting table so that a continuous record is obtained during the change of position it is seen that the alteration in respiratory level is immediate.

The most obvious explanation of this change is that the weight of the abdominal viscera presses the diaphragm upwards in the supine position, thereby reducing the volume of air in the lungs. Radiological examinations by Hurtado and Fray (9) leave no doubt that such an upward shift of the diaphragm in the resting (end-expiration) respiratory position actually occurs in recumbency, and this largely accounts for the alteration in respiratory level. Fig. 2 shows a tracing taken from a subject in the sitting position. When a towel round the abdomen was tightened air was squeezed from the thorax, and a position of greater expiration was adopted. The original level was reached immediately on releasing the pressure.

While this simple mechanical explanation appears acceptable it should be noted that recent physiological work has indicated that chemical and reflex factors may play a part in determining the respiratory level. Verzar (16) showed that when animals were made hyperpnoeic by such measures as CO₂ inhalation, oxygen lack, etc., the chest adopted an increased inspiratory

position. Greene (16) has shown that the respiratory level also moves towards an inspiratory position during the hyperpnoëic phase of Cheyne-Stokes breathing. It has been established (11) (12) that in the upright position there is an increase in pulmonary ventilation, and it is therefore possible that the increased ventilation induces a shift towards the inspiratory position by chemical or reflex means.

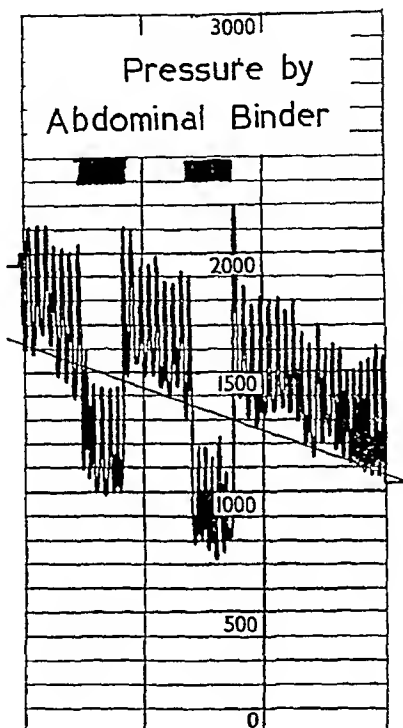


Fig. 2. Effect on respiratory tracing of increased pressure on the abdomen. Tracing read from right to left. Abdominal pressure applied during the times marked by rectangles.

It is unlikely that chemically induced changes in respiratory level are responsible for the postural shift, which is too immediate in onset to be determined by changes in blood gases. One of us (McM.) has attempted to produce alterations in the resting respiratory level by inducing hyperpnoëa by CO_2 inhalation and oxygen lack, but with no significant result.

Hess (1932) has described alterations in resting diaphragmatic tone when vagal influences from the lung are abolished or modified, but one of us (McM.) has failed to produce any appreciable shift of the respiratory level in cats by severing the vagi. Other reflexes influencing respiration such as the carotid sinus reflex have also been examined both in animals and man. In two subjects lowering of pressure in the carotid sinus by amyl nitrite

caused a slight shift of the respiratory level towards inspiration, but in four the results were negative. Slight changes in a similar direction were also produced in cats by lowering the carotid sinus pressure, but the results were slight and inconstant.

The conclusion is therefore that the shift in respiratory level associated with change in posture is almost entirely accounted for by pressure of the abdominal viscera on the diaphragm. The various alterations in respiratory level induced by chemical and reflex means seem to be too slight and inconstant to play any important part.

The diminution in the total lung volume.

The total volume of air in the fully expanded lungs invariably diminishes on assuming the flat position, the diminution averaging 340 c.c.. In considering the mechanism involved it is necessary to study the changes which take place in the size of the thoracic cage with change of position. It has already been shown by Hurtado and Fray (9) that the resting position of the diaphragm is considerably altered in recumbency, but there is still some doubt as to whether the chest expansion in full inspiration is as complete in the recumbent as in the upright position.

For radiological measurements ten subjects were taken who were known to be sufficiently co-operative to achieve and hold the position of full inspiration while a radiographic exposure was made. The films were placed posteriorly to avoid the inspiratory difficulty associated with the prone position. It was found, however, that the films so taken were not all strictly comparable; with the change from the upright to the recumbent position there may occur an alteration in the inclination of the longitudinal thoracic axis to the incident X-ray beam (referred to subsequently as thoracic "tilt"). To observe the effect produced by such thoracic tilting radiograms were taken in one subject standing upright who leaned slightly forward and slightly backward. Photographs were also taken keeping the thoracic axis unchanged but altering the angle of the incident beam by raising and lowering the tube 7.5 cm.. Examination of these films showed that leaning backward produced no significant change in radiological chest area, but, leaning forward, the area rose from 103.3 to 113 sq. in.. Lowering the tube produced only a slight decrease in area, while raising the tube increased the area from 103.3 to 106 sq. in.. The observed changes in area were accounted for by alterations in the apparent level of the dome of the diaphragm. It is notable that a forward tilt of the chest produced a much greater effect on the area than shifting the tube through a range of 15 cm.. Careful centring of the tube was therefore not in itself sufficient to ensure comparable films in the upright and recumbent positions because slight changes in the forward inclination of the thoracic axis may produce very distinct alterations in the radiological chest area.

In order to avoid these parallax errors it was necessary to ensure that the inclination of the thorax to the incident beam remained constant. This could be achieved if, in each pair of films, a given fixed point on the front of the thorax was superimposed on a second fixed point on the posterior wall of the thorax. A measurement may be taken from the mid-line to the point at which the shadow of the upper border of the second rib in front crosses the lower border of the third rib behind (Fig. 3). Any forward tilting of the



Fig. 3. Diagram of second and third ribs as seen in a radiograph. The dotted line indicates the measurement called II-III rib crossing distance in Table III.

thorax would be disclosed by an increase in this distance and, conversely, increased lordosis would diminish the measurement. If this distance remains unchanged in the radiographs taken in the upright and recumbent positions, then it is obvious that there has been no change in the tilt of the thorax. In practice it is better to take a measurement from the above point of rib crossing to the corresponding point on the opposite side. This obviates the difficulty of measurement from the mid-line, and ensures greater accuracy. When this was done in the present series it was found that five out of ten pairs of radiographs were strictly comparable.

The radiological chest areas in these subjects are shown in Table III. It is seen that the average area in the upright position is 112.3 sq. ins. while

TABLE III.

Radiological chest areas in upright and supine positions.

Subject No.	II-III rib crossing distance (cms.)		Radiological chest area (sq. ins.)	
	Upright.	Supine.	Upright.	Supine.
11	12.5	12.3	105.0	109.0
3	18.8	18.8	111.4	107.9
4	17.0	16.8	107.6	108.3
17	19.0	19.1	120.5	121.9
19	19.4	19.6	117.0	115.9
		Average	112.3	112.6

in the supine position it is 112.6 sq. ins. There is thus no significant change in the projected area of the chest cavity. The measurement of area takes into account both the level of the dome of the diaphragm, and the lateral expansion of the chest. Measurements made of the antero-posterior chest diameters in the upright and supine positions also failed to reveal any significant difference.

It is thus apparent that chest measurements have failed to reveal any change in the size of the thoracic cavity when the recumbent position is adopted. The only alternative explanation of the diminution in total lung volume is therefore encroachment on the pulmonary air space by an increased amount of blood in the thorax. It is now generally accepted that the output of the heart normally increases by 25-30% in the recumbent position. This increase in output is the result of an increased venous return to the right heart. Correspondingly, to maintain an increased output of the left ventricle the pulmonary venous pressure must increase *pari passu*. It is not surprising therefore to find evidence that the pulmonary vessels become more congested in the recumbent position. Weiss and Blumgart (17) have calculated that the average volume of blood normally present in the human lungs is just under one litre. A postural change of one-third of a litre in this volume is therefore not an unreasonable quantity when we consider the simultaneous 30 per cent. change in cardiac output.

The diminution of residual air.

While the lung volume decreases by 340 c.c. the vital capacity decreases by 190 c.c. on the average. Correspondingly it is found that the residual air decreases on the average by 150 c.c.. In 7 out of 25 subjects the residual air underwent an apparent increase, but this is probably accounted for by accidental errors in the estimation, as the standard error of the measurement of each individual difference is ± 0.13 litre.

If we accept the hypothesis that the total lung volume is encroached upon by an increased blood content of the capillaries, we should naturally expect to see a comparable encroachment on the residual air. The fact that the residual air does not decrease to the same extent suggests that maximal expiration is not so effective in reducing the size of the thoracic cage in the recumbent position.

The answer to the problem created by this discrepancy will depend on an exact knowledge of the factors which limit forced expiration in man. As yet these factors are not understood. It is important to note, however, that when the lungs become markedly congested in heart failure, the volume of the residual air tends to remain practically unchanged (3) (14). It is thus apparent that even severe congestion may not diminish the residual air appreciably. Lack of parallelism between the diminution of total lung volume and of residual air is therefore not a valid argument against the hypothesis that the total volume is encroached upon by congestion in recumbency.

SUMMARY.

Postural changes in lung volume and its subdivisions have been studied and the following conclusions are reached :—

1. In the recumbent position the total volume of air in the fully expanded lungs decreases by 340 c.c. on the average. This decrease is unassociated with any measurable diminution in the size of the thoracic cage. It is therefore presumably due to an increased congestion of the pulmonary vessels in the recumbent position.

2. The functional residual air undergoes an average decrease of 780 c.c. in recumbency. This is largely a passive effect due to an upward shift of the diaphragm. Chemical and reflex alterations of diaphragmatic tone have been considered, but have not been found to play any significant part.

3. The residual air decreases by 150 c.c. The failure of pulmonary congestion to encroach on residual air to the same extent as on the total lung volume is discussed.

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THE EFFECT OF ŒSTROGENS ON THE URINARY CREATININE OF CASTRATE AND MENOPAUSAL WOMEN.

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It is now generally accepted that following castration the anterior pituitary gland secretes increased quantities of gonadotropic principle. Engle (4) showed that the gonadotropic potency of the rat's pituitary was increased following castration, and the work of Martins (22) and Kallas (16) in experiments on animals united in parabiosis made it clear that the gonadotropic principle was not only stored in greater amounts in the pituitary but was also secreted in excess. Up to the present time there appears to be no reliable information regarding the amount of gonadotropic hormone that is secreted by castrate and menopausal women. Henderson and Rowlands (13) have shown that the human anterior pituitary, obtained after death, contains greater amounts of gonadotropic principle after the menopause than before, and that the gonadotropic potency increases with age. There is also an increased excretion of this principle in the urine of women past the menopause and castrated subjects of both sexes (11) (21) (30) (33). In the blood of similar subjects Fluhmann (6) states that there is increased gonadotropic activity. The effect of injection of the Œstrogens on the gonadotropic activity of the anterior pituitary has been extensively studied in animals. Allen (1) reported a depressant effect on the gonads of immature monkeys following the injection of ovarian extracts, and similar results have been found in dogs (18), in rats (20) and in mice (10). The results of these researches have been reviewed by Deaneley (5) who showed a depression of the gonads and enlargement of the adrenals and pituitary gland following the implantation of tablets of Œstrogens under the skin of rats and mice. In man, Frank and Salmon (8) and Jones and MacGregor (15) reported a disappearance of a follicle stimulating principle from the urine of women past the menopause following the injection of large doses of the Œstrogens.

In a previous communication (28) it was shown that the effect of injection of large doses of the Œstrogens into subjects with acromegaly could

* The authors acknowledge the permission of the Chief Medical Officer, London County Council, to publish these investigations.

be studied by following the excretion of creatinine in the urine. Instead of the constant amount from day to day that is found in the normal subject, the daily quantity of urinary creatinine that is excreted in acromegaly tends to fluctuate widely, and on occasions is greatly in excess of the amount that would be expected for normal individuals of the same size (26). The effect of injecting large doses of the oestrogens was found to cause the amount of urinary creatinine to become constant at a lower level, which would be compatible with the amount that might be excreted by a normal subject of the same size. On ceasing injection in these cases of acromegaly the urinary creatinine again became excessive and fluctuating. It was suggested that one function of the overactive anterior pituitary in acromegaly could be depressed by large doses of the oestrogens. Evidence (27) was also obtained as to which function of the pituitary was involved, since the injection of a pituitary gonadotropic extract into normal human subjects caused an immediate excess of creatinine to be excreted in the urine, while growth and thyrotropic extracts and those gonadotropic substances that are prepared from pregnancy urine and pregnant mare's serum had no effect on the urinary creatinine. It was therefore decided to study the effect of the oestrogens on the urinary creatinine of castrate and menopausal women, since these are states that are believed to have a physiological overactivity of the pituitary, though the overactivity is probably considerably less than that caused by the eosinophil tumour in acromegaly.

Methods.

The subjects investigated were at rest in bed in hospital. Their diet was prepared by the hospital dietitian (Miss Simmonds) and was creatine-free. Urine was collected over twenty-four hour periods under toluene and placed immediately in the ice-chest on the completion of each period. Mistakes in the collection of urine occurred on a number of occasions and the estimations for these days were discarded. Urinary creatinine was estimated by Folins' (7) method. Oestrogens were administered intramuscularly in the form of oestradiol benzoate (Dimenformon, Organon Laboratories) in doses of 100,000 I.B.U. daily except in two cases where diethylstilboestrol was injected in 10 mgm doses daily.

Results.

Normal subjects. The effect of the injection of oestradiol benzoate was investigated in 9 subjects, young adults without disease of the endocrine system; 3 were males and 6 females who were menstruating normally. In 7 of these normal subjects no effect was seen on the urinary creatinine (Fig. 1). In two subjects one male (Fig. 2) and one female there appeared to be a slight increase of the amount of creatinine excreted during the period of oestrogen injection as compared with the control period. In no case was any diminution of urinary creatinine observed during or following the period of injection.

Menopausal subjects. Seven women with menopausal symptoms were investigated. Every subject was complaining of hot flushes and the shortest period of amenorrhœa was three months and the longest four and a half years. Five of the subjects gave similar results when injected with œstrogens (Fig. 3). During the control period the daily urinary creatinine tended to fluctuate without ever exceeding the limits that might be expected for a

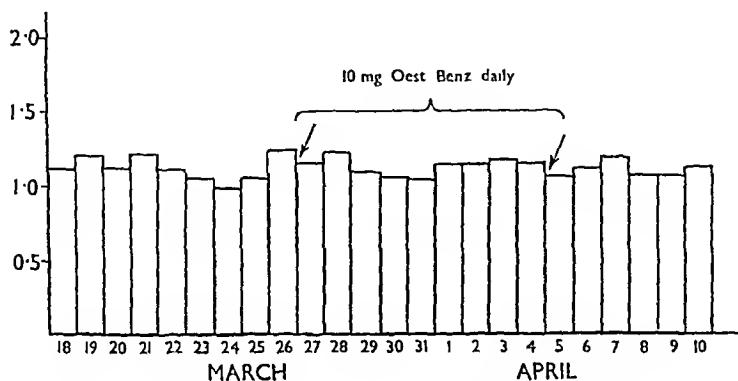


Fig. 1. Urinary creatinine in g. per 24 hours. Normal male.

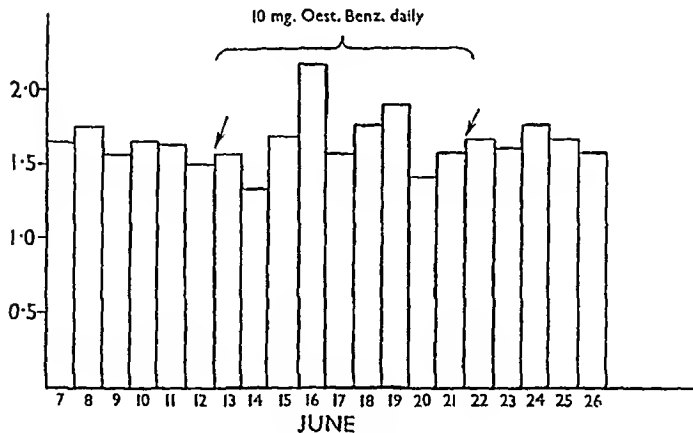


Fig. 2. Urinary creatinine in g. per 24 hours. Normal male.

young adult subject of the same size. On injecting œstrogens, however, the daily creatinine excretion became constant at a level corresponding to the lower daily quantities which had been observed during the control period. At the end of ten days injections were stopped and in each case the amount of creatinine excreted again began to fluctuate. Two cases showed very little fluctuation during control period and when injected with œstrogens showed only a slight fall of 0.1 to 0.2 grams of creatinine a day. The effect

of injection of diethylstilboestrol in doses of 10 mg. daily appeared to be identical to the effect obtained by the injection of 100,000 I.B.U. of oestradiol benzoate.

Castrate subjects. Two women, one castrated 4½ and the other 25 years previously, were also investigated. The results obtained in the 25 year castrate are shown in Fig. 4. The effect of injection of oestradiol benzoate

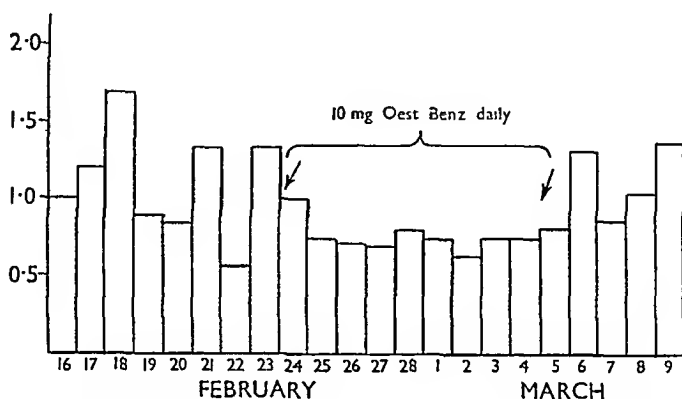


Fig. 3. Urinary creatinine in g. per 24 hours. Menopausal female.

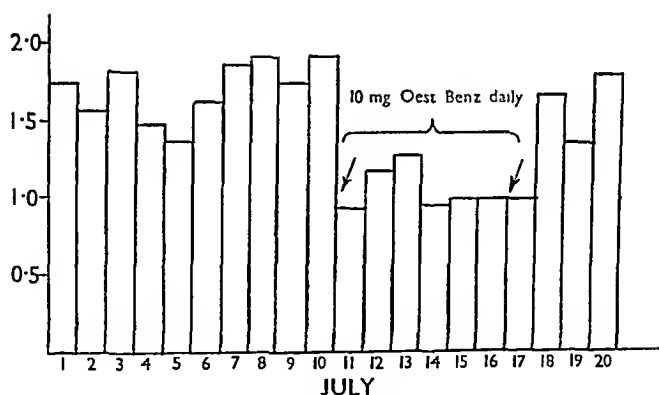


Fig. 4. Urinary creatinine in g. per 24 hours. Castrate female

on the urinary creatinine in these two castrates appeared to be even more striking than that observed in the menopausal women. During the control period the amount of creatinine found in the urine was greater than the upper limit of the calculated normal and was excreted in a more constant amount from day to day than in the menopausal subjects. There was a sharp fall on starting injection of oestradiol benzoate and an equally sharp rise on ceasing injection.

Discussion.

From the results given above it is seen that the amount of urinary creatinine excreted daily by castrate and menopausal women may be depressed by injecting large doses of the œstrogens. No such depression of creatinine excretion was observed in the normal controls though two subjects in this group showed a slight rise of urinary creatinine while being injected with œstrogens. A similar rise in urinary creatinine has been observed in normal rabbits following the administration of testicular extracts (2) but no really adequate explanation for this phenomenon has been forthcoming. The administration of the œstrogens or androgens to mammals with an intact endocrine system may well produce diverse and conflicting results. Thus Freed, Greenfield and Soskin (9) have shown that testosterone propionate has a biphasic action in female rats, small doses inhibiting while larger doses stimulated the gonads probably through the pituitary. It is possible that the rise of creatinine observed in certain normal subjects might imply an actual stimulation of the anterior pituitary by this dosage of œstrogens.

The effect of œstrogens on the urinary creatinine of menopausal women is very similar, but in a lesser degree, to the effect previously observed in acromegaly and it is suggested that in both cases it signifies a depression of an overactive pituitary function. It is not surprising that two subjects out of seven should show only a very slight effect. On clinical grounds alone it may be observed that there is considerable individual variation in the severity of menopausal symptoms; indeed some women appear to escape them altogether. It is not suggested, however, that the symptoms of the menopause are necessarily due to an overactive pituitary since objective proof is still lacking, though in the present state of our knowledge it would appear likely that excess pituitary function is a factor.

The results in castrates are similar and even more striking than those obtained in the menopausal subjects and are compatible with experiments done previously in rabbits (29). Ovariectomy of adult female rabbits led to a 16% increase in the excretion of urinary creatinine commencing at least five months after operation and the injection of saline suspension of ovary and ovarian extracts produced a fall in the high urinary creatinine of such castrates. Other workers have not agreed with these results. Kochakian and Murlin (17) in dogs, Sandberg, Perla and Holly (25) and Pizzolato and Beard (23) in rats found no increase in urinary creatinine following castration. In explanation of these apparently divergent results the workers on dogs and rats did not conduct their observations over long periods and it has already been stressed that five months or more may elapse after castration of female rabbits before a rise of urinary creatinine is found (29). Another possible explanation is that there is a species difference. However, man appears to follow the rabbit in showing an excess of urinary creatinine following castration, and a return to normal levels on injecting the œstrogens. Our results also agree with the work of Frank and Salmon (8) and Jones

and MacGregor (15) who observed the disappearance of gonadotropic hormone from the urine of similar subjects following the injection of oestrogens. On the other hand Heller and Heller (12) failed to find any diminution of the urinary gonadotropic substance of post-menopausal women following treatment with oestrogens. A simple explanation of their results is afforded when the dosage of oestrogens used by various workers is examined. Frank and Salmon used 4,000 to 22,000 R.U., Jones and MacGregor 50,000 M.U., while ours has been 100,000 I.B.U. daily throughout these investigations. Heller and Heller gave 8,000 I.U. by mouth daily and in two cases 10,000 I.U. daily by intramuscular injection. These doses are considerably smaller than the other workers quoted above, and we have unpublished evidence that in acromegaly small doses of the oestrogens will not alter urinary creatinine, thus it would appear likely that the higher dosage is necessary to demonstrate an effect on the anterior pituitary in man.

Although the view is put forward that the effect of oestrogens on the urinary creatinine of acromegaly and castrate and menopausal women is probably due to a depression of an overactive pituitary function, yet diminished excretion products in the urine do not necessarily imply a diminished activity of the cells of the anterior pituitary. The high gonadotropic content of the anterior pituitary of post-menopausal women can be diminished by prolonged oestrogen administration even to the point where it is undetectable (24). Yet this shows only that the content of the gland is less and may be due either to an increased or decreased function of the pituitary cells. Severinghaus (32) has taken the view on cytological grounds that the oestrogens stimulate rather than inhibit the anterior pituitary and with small doses the evidence (5) (14) (19) (31) suggests that secretion of gonadotropic hormone may be increased. The bulk of the work on castrates, however, seems to show that pituitary activity increases when the gonads are removed and that this increased activity may be inhibited by large doses of the oestrogens.

SUMMARY.

1. The effect of the injection of the oestrogens, 100,000 I.B.U. of oestradiol benzoate or 10 mg. of diethylstilboestrol, on the daily urinary creatinine excretion was studied in normal men and women and menopausal and castrate women.

2. Seven out of 9 normal subjects showed no alteration of urinary creatinine during oestrogen injection; two showed a slight rise.

3. In 5 of 7 menopausal women oestrogens caused a depression of urinary creatinine and a greater depression was caused in two castrate women.

4. It is suggested that the excessive function of the anterior pituitary in castrate and menopausal women may be inhibited by large doses of the oestrogens.

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SOME PAINFUL JOINT CONDITIONS AND THEIR RELATION TO OSTEOARTHRITIS.

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School, London.)*

MANY clinicians (1) (3) (6) (7) (9) have suggested that "Joint pains" may sometimes arise from localised lesions in the joint structures or in the surrounding muscles. From an experimental study (5) of the distribution of pain arising from the somatic deep structures, we know that this pain is always felt diffusely, and that in the limbs it is often felt maximally in the region of the joints. It thus becomes increasingly clear that a "painful joint" may result from quite a localised stimulus situated in one of the joint structures, or in some more distant structure such as muscle.

With this in mind I decided to search for the source of pain and disability in various obscure joint conditions, and to demonstrate the structure involved by anæsthetising this locally with novocain and abolishing the symptoms. Various joints were investigated in this way, but for more thorough study the knee and hip were chosen.

The procedure was as follows: All patients complaining of painful knees or hips were examined. Those presenting some well recognised disease such as tuberculosis, pyogenic or rheumatoid arthritis, or internal derangement of the knee, were rejected,† as were patients complaining of vague pains, but in whom no disability could be detected. In the remaining cases I tried to find the source of pain and disability, and where practicable this was confirmed by local anæsthesia. Both knees or hips were always X-rayed to determine the presence of any bony change.

Findings in the knee.

Of patients complaining of painful knees 55 were investigated in this way. Among these cases three distinct clinical pictures occurred repeatedly. These may conveniently be described as cases with ligamentous or muscular pain, cases with synovitis, and cases with a disordered joint mechanism.

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† Patients presenting osteoarthritis in the X-ray picture were not excluded for reasons that will become apparent.

Ligamentous and muscular pain. This group consisted of 39 cases. The outstanding feature was pain. This was a continuous deep ache, aggravated by movement and often causing the patient to limp; there might also be a sense of tightness or stiffness at the back of the knee. The knee movements were always limited to some extent; flexion being limited by from 20° to 140° , and rotation of the tibia on the femur was usually painful, though extension was often full. The joint contained no excess of fluid, and presented little if any synovial thickening. Wasting of the quadriceps was slight or altogether absent, and muscle spasm was not observed, though some voluntary guarding was usual against movements that were known to be painful. On palpation one or more acutely tender spots were found in the muscles or ligaments surrounding the joint, and anæsthetising these spots with 2 to 20 c.c. of 1% novocaine relieved the pain and disability completely or almost completely in all cases; so that the source of pain in this group is clearly established.

TABLE I.

Showing the age and the degree of osteoarthritis present in 53 patients with painful knees due to ligamentous and muscular "strains" (L), synovitis (S) and disordered joint mechanism (D).

Age.	DEGREE OF OSTEOARTHRITIS.				
	None.	Slight.	Moderate.	Considerable.	Gross.
20-29	LLLL SSS	—	—	—	—
30-39	LLLL SS	L	—	—	—
40-49	LLLLL S	LLL	L	L	—
50-59	LL	LLLLLL S	LL	L S	D
60-69	LL	L SS	LLLL	LL	DD
70-79	—	—	—	D	—

The clinical picture varied slightly with the source of pain. Thus muscular pain was of constant intensity, while ligamentous pain was largely provoked by movement. The pain was distributed in one of three recurring patterns, and each pattern arose from characteristic tender spots, the usual position and frequency of which are shown in Fig. 1. In lesions of the capsule

on the medial side of the knee flexion was moderately limited (20° to 60°) and pain on rotation was prominent, while in lesions of the quadriceps insertion flexion was greatly limited (60° to 140°) and rotation was painless. Lesions of the lateral part of the capsule were characterised by pain distributed widely over the outer side of the leg with little or no area of maximal pain in the region of the knee.

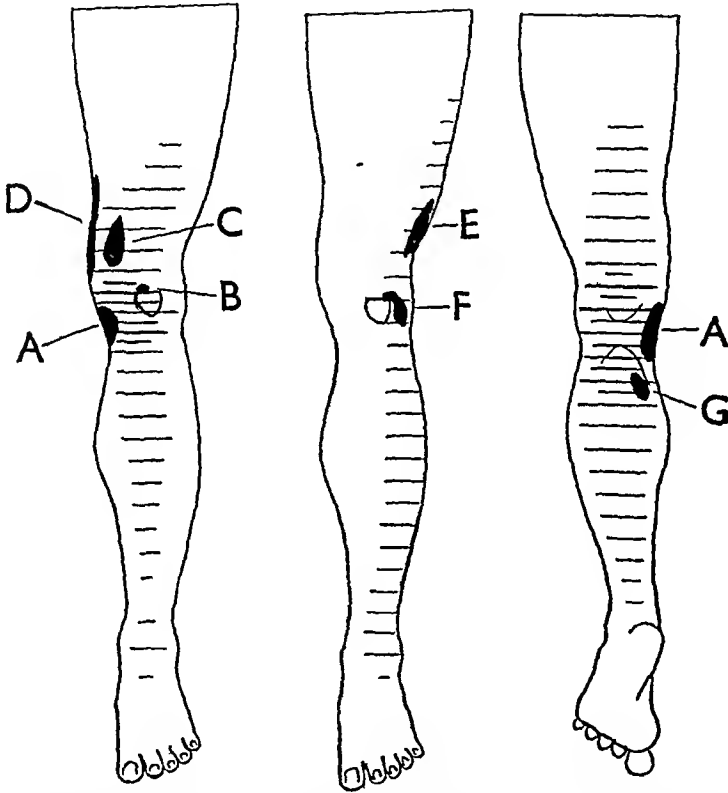


Fig. 1. Shows the distribution of pain (hatching) and its source of origin (black) in 39 cases of ligamentous or muscular knee pain. The numbers indicate the number of cases in which each source of pain was demonstrated. A (23) medial part of capsule. B (7) quadriceps insertion. C (6) vastus medialis. D (2) sartorius and adductor muscle. E (4) vastus externus. F (4) lateral part of capsule. G (3) medial head of gastrocnemius.

At the time of examination symptoms were bilateral in 4 cases and unilateral in 35 though a few of these cases had suffered from previous attacks of pain in the other knee. The duration of symptoms varied from 1 week to 5 years, 1 to 3 months being usual. A definite history of trauma was obtained in only 17 cases though the clinical picture of the remaining 22 cases differed little from that of the 17 "traumatic" cases which were typical "sprains" of the knee. The age distribution and the radiological

findings in this group are shown in Table I. The following 3 cases are typical.

Case 1. S.H., a police constable of 33 years. Six weeks previously he sprained his left knee in a motor accident. He had since suffered from pain and stiffness of the left knee. The pain was felt over the outer side of the knee and calf down to the ankle; it was continuous but aggravated by movement.

This knee showed no excess of fluid, no synovial thickening, and no wasting of the quadriceps. Knee flexion was limited by 80° and rotation of the tibia on the femur caused pain, but extension was full. There was a tender spot in the joint capsule over the outer side of the knee. Anæsthetising this spot with 4 c.c. of novocaine resulted in a painless knee that could be moved through a full range. The other knee appeared normal. X-ray showed both knees to be normal.

Case 2. A.E., a housewife of 58 years. For 3 weeks she had suffered from severe continuous pain in the left leg which was little affected by rest or movement. No history of trauma could be elicited. The pain was felt maximally over the front of the knee, but it spread up the thigh and down the shin to the ankle.

This knee showed no excess of fluid, no synovial thickening, and no wasting of the quadriceps. Knee flexion was limited by 40° but extension and rotation were full and painless. There were several acutely tender areas situated over the vastus medialis, sartorius and adductor muscles. These were anæsthetised with 20 c.c. of novocaine. This abolished pain completely, but there was still 20° of limitation of flexion. In the right knee flexion was limited by 20° , otherwise it was normal. X-ray showed moderate osteoarthritis of both knees; the changes being symmetrical.

Case 3. S.R., a housewife of 66 years. Three months previously she tripped and sprained her right knee. She had since suffered from continuous pain in the right knee aggravated by exercise. Previously she had had no trouble with her knees.

The knee showed no excess of joint fluid, but there was slight synovial thickening. Knee flexion was limited by 90° and extension by 5° and rotation was painful. There was an acutely tender spot over the medial part of the joint capsule and another on the upper border of the patella. These were anæsthetised with 10 c.c. of novocaine. This abolished pain and the knee could now be moved through a nearly full range, flexion being only limited by 10° .

The left knee also showed slight synovial thickening and 10° limitation of flexion, but it was quite painless. The quadriceps was poorly developed in both legs. X-ray showed considerable osteoarthritis of both knees; the changes being symmetrical.

Synovitis. In this group there were 10 cases. The outstanding feature was unilateral swelling outlining the limits of the synovial sac. This swelling resulted from excess of synovial fluid in the joint in 5 cases, blood in the joint in 3 cases, and thickening of the synovial tissues in 2 cases. Some synovial thickening could also be made out in 4 of the other cases after the blood or fluid had been aspirated. Atrophy of the quadriceps was prominent in most cases and wasting was conspicuous in all except one case in which the swelling had only been present for 3 days. Pain was variable, often slight, the chief symptom being a sense of insecurity and weakness in the affected knee. The knee movements were limited both in flexion and extension but rotation was free and painless.

In the cases with excess fluid or blood in the joint, the symptoms were abolished or greatly relieved by aspiration so that the clinical picture of this group probably results from the synovial swelling. In 5 cases the synovitis was associated with ligamentous or muscular lesions similar to those already described. A clear history of trauma was obtained in all except one of these cases. The age distribution and the radiological findings in this group are shown in Table I. The following cases are typical.

Case 4. A.S., a housewife of 53 years. Three weeks previously her right knee "gave way" while she was trying to jump onto a bed. The knee had since been swollen and felt weak and unstable, with slight pain at the back of the knee.

The knee showed an excess of joint fluid, but no synovial thickening, and the quadriceps was wasted and atonic. Flexion was limited by 40° and extension by 5° but rotation was painless. No tender spots could be found. 40 c.c. of clear fluid were aspirated from the joint. After this the knee could be moved through a full range and felt normal. The left knee appeared normal. X-ray showed slight osteoarthritis in both knees; the changes being symmetrical.

Case 5. A.H., a man of 57 years. Six days previously he fell and struck his left knee on the corner of a box. Three days later the knee became stiff and swollen and he had great difficulty in walking because the knee "let him down." Pain was slight.

The left knee on examination showed a great excess of joint fluid, but no wasting of the quadriceps could be detected. Flexion was limited by 140° and extension by 10°, and there was a small tender spot over the upper border of the patella. 100 c.c. of bloody fluid was aspirated from the joint. After this the knee felt much stronger and flexion was only limited by 80° and extension was full. The tender spot near the patella was then anaesthetised with 1 c.c. of novocaine. Afterwards flexion was only limited by 40° and the knee was painless.

The right knee showed 20° painless limitation of flexion but no other disability. X-ray showed considerable osteoarthritis of both knees; the changes being symmetrical.

Disordered joint mechanism. In this group there were 4 cases. They all presented some serious disorder of joint mechanism. The symptoms were of long standing (5 to 20 years), and were bilateral in 3 of the 4 cases. As the clinical picture varied in these cases they will be described separately.

Case 6. T.A., a retired ostler of 68 years, who had suffered from increasing pain in the right leg for 20 years. At rest he was fairly comfortable, the pain being brought on by moving the knee.

The knee showed no excess of joint fluid, and little synovial thickening, but there was considerable wasting of all the muscles in the right leg. The range of knee movements was nearly full, but the movement was irregular and accompanied by tonic and clonic spasm of all the muscles in the thigh, calf and shin; pain at rest was slight, but increased rapidly as the movement continued. There were many tender areas in the various muscle groups surrounding the knee and also over the joint capsule. These regions were infiltrated widely with large quantities of novocaine, but this had little effect on the pain, and the muscle spasm occurred as before in all muscles that were not paralysed by the anaesthetic.

A similar condition was present in the left hip, but the left knee appeared normal. On X-ray the right knee showed gross osteoarthritis with loss of joint space and much deformity of the ends of the bones, but little osteophyte formation; the left knee showed only slight changes of a similar nature.

Case 7. C.R., a clerk of 52 years, had suffered from increasing pain in both legs for 10 years. He suffered little pain at rest, but moving his knees produced severe pain and his legs became quite "fixed."

On examination there was considerable wasting of the muscles of both legs. The range of knee movements was nearly full, but the movements were jerky and accompanied by severe tonic and clonic spasms of the leg muscles. There was no excess of joint fluid and no synovial thickening. Tenderness was widespread in the knee and leg muscles. The right leg was more affected than the left. X-ray showed osteoarthritis in both knees with loss of joint space, much irregularity and deformity of the ends of the bones and much osteophyte formation. The changes were more advanced in the right than the left knee.

Case 8. A.M., a housewife of 64 years, had suffered from increasing pain in both legs for over 10 years. The pain was continuous, aggravated by movement, and she could only walk with great difficulty.

On examination both knees showed a fixed flexion deformity of 30° and movement could only be obtained through a range of 70°. Both quadriceps muscles were wasted but there was no excess of joint fluid and little synovial thickening. Muscle spasm was not observed. There was widespread tenderness of both quadriceps muscles and also over the joint capsule. X-ray of both knees showed gross osteoarthritis with much narrowing of the joint space and deformity of the ends of the bones; there were very large interlocking osteophytic outgrowths, the changes being symmetrical.

Case 9. M.T., a housewife of 75 years, had suffered from increasing pain in both knees for 10 years. The pain was continuous but aggravated by moving the knees; and for the last 6 months she had been unable to walk. She was a very heavy woman who on examination showed bilateral genu varus and some wasting of both quadriceps muscles. The range of knee movements

was nearly full; there was considerable lateral mobility in both knees which could also be hyperextended through 20°. There was no excess of joint fluid and little synovial thickening. The joint motion was smooth and unaccompanied by much spasm. There were several tender spots in the quadriceps and over the joint capsule. Anæsthetising these spots by an extensive novocaine infiltration rendered the knee temporarily painless. X-ray showed considerable osteoarthritis of both knees with narrow joint space and deformity of the ends of the bone, but little osteophyte formation; the changes were symmetrical.

These four cases present the classical clinical picture of osteoarthritis; they were included in this investigation for reasons that will become apparent when the radiological findings are discussed.*

Radiological findings in the knee. In all of the 55 patients, both knees were X-rayed. The only bony changes that were noticed were those of osteoarthritis, and these were found in more than half of the cases. Figs. 2-9 illustrate the several degrees of osteoarthritis recognised. With the exception of Cases 6 and 7, these changes when present, were symmetrical, occurring equally in both knees. The age incidence of these changes and their relation to the clinical groups just described, is shown in Table I.

It will be seen that the incidence and degree of osteoarthritis in the whole group of cases increases steadily with advancing age, and differs little from the incidence of such changes described by Heine (4) and others (2) (8) in routine autopsies. It will also be noticed that the four cases with disordered joint mechanism and much deformity of the bone ends, all show gross or considerable osteoarthritis; while the cases with synovitis, or ligamentous and muscular pains, show varying degrees of osteoarthritis or none at all. The significance of these findings will be discussed later.

Findings in the hip.

The hip joint lies deeply embedded in the muscles that move it, so that it is much more difficult to examine than the knee. For instance the presence of synovial thickening cannot be made out clinically, and it is very difficult to decide whether pain is arising from the joint capsule or the overlying muscle.

However, in 11 cases an attempt was made to find the source of pain and disability and to confirm this with local anæsthesia. The findings in the hip differed from the findings in the knee. Of the 11 cases only 3 could be classed as ligamentous or muscular pains and in these osteoarthritic changes were either absent or slight and symmetrical.

In the remaining 8 cases there was a considerable disorder of joint mechanism. In all of these the hip movements were much limited and the limb more or less deformed. In 4 cases the movements were also irregular and accompanied by widespread tonic and clonic muscle spasm. In the cases without muscle spasm, novocaine infiltration of tender areas in the

* There were also two cases which could not be placed in the clinical groups just described. One of these cases presented a semimembranous bursa distended with fluid, and his symptoms were relieved by aspirating the bursa. The other case presented pain arising from the gastrocnemius, and a painless limitation of knee movements; on X-ray the soft tissues around the knee showed extensive calcification.

muscles and ligaments surrounding the hip relieved pain for a variable period, but the limitation of movement remained. In the cases with muscular spasm, widespread infiltration of tender areas only reduced pain and spasm. X-rays of the hips in these 8 cases all showed considerable or gross osteoarthritis. Except in one case (Case 11), in which the symptoms were bilateral, these changes were chiefly confined to the side of the symptoms. The following 3 cases illustrate these points.

Case 10. A.H., a housewife of 56 years, had suffered from pain in the left leg for 6 months. The pain was felt diffusely in the buttock and down the back of the thigh and calf, it was continuous but aggravated by moving the hip.

The left hip on examination showed painful limitation of abduction, adduction and rotation, and straight leg raising could be obtained to 100° on the right side and 85° on the left. No muscular spasm or wasting could be detected. There was acute tenderness of the gluteal muscles just above the great trochanter. This tenderness and the pain were abolished by 25 c.c. of novocaine and the hip could then be moved through a full range. The right hip appeared normal. X-ray showed both hips normal.

Case 11. A.G., a baker of 38 years, was first seen 2 years ago. At that time he had suffered from pains in both legs on and off for 7 months and continuous pain in the left leg for 1 week. The pain was distributed widely in the thigh and calf with an area of maximal pain in the knee.

The left hip on examination showed considerable limitation of abduction and rotation, and some limitation of flexion and extension. No muscle spasm or wasting could be detected. There was tenderness of the gluteal muscles above the great trochanter. This tenderness and the pain were abolished by 20 c.c. of novocaine, but the hip movements remained limited as before. The right hip was painless but showed a similar limitation of movement. X-ray showed considerable osteoarthritis of both hips with coxa-plana. Two years later he returned with a similar painful condition, this time in the right leg. This was again relieved by anaesthetising a tender area in the gluteal muscles.

Case 12. W.S., a retired shop assistant of 76 years. For 4 years he had suffered from increasing pain and stiffness of the right leg. The pain was felt widely in the buttock, thigh, and knee and was greatly increased by movements of the right hip.

The right hip on examination showed a fixed flexion and rotation deformity, and all movements were greatly limited. What movement there was was bumpy and irregular, and accompanied by tonic and clonic muscle spasm which was maximal in the sartorius and adductor muscles. The buttock and leg muscles were much wasted and there was widespread tenderness in the buttock, back and thigh muscles. Extensive infiltration of these tender areas reduced pain slightly but the hip movements remained unaltered, and muscle spasm persisted. The left hip was painless, and showed only slight limitation of movement. X-ray of the right hip showed gross osteoarthritis with loss of joint space and great bony deformity. The left hip showed only moderate osteoarthritis.

The difference between the findings in the knee and hip probably result from the anatomical differences between the two joints. Thus the knee is a mechanically unstable joint, and any stress applied to the leg is likely to strain the muscles and ligaments that support it. The hip on the other hand, is extremely stable, and in practice strains of the hip are rare, and symptoms arising from this region are usually associated with a considerable disorder of the joint mechanics.

Discussion.

The observations described raise two distinct points for discussion. One arises from the clinical, and the other from the radiological findings.

Clinical findings. In the painful joints investigated, the clinical picture appeared to be related to the source of pain, and on this basis 3 distinct clinical groups have been described. These groups present certain features

which are of general interest in connection with joint disease. It has generally been thought that in joint disease pain, muscle spasm, and wasting are directly related to each other, but in the cases reported here this was not so. For instance, in the cases suffering from ligamentous or muscular "strains" pain was prominent and often disabling, but muscular wasting was slight or absent; and although there was some guarding against movements known to be painful, involuntary muscle spasm was not observed. In the cases of synovitis, on the other hand, pain was less but wasting of the quadriceps was conspicuous, and this muscle was flaccid and atonic.* The cases with disordered joint mechanism were again different. In them pain was variable but there was always considerable wasting of all the muscles in the affected limb, which was generally disused. A number of these cases also presented a remarkable form of tonic and clonic muscle spasm. The spasm rarely involves a muscle as a whole, but consists of irregular contractions of groups of muscle fibres thus resembling a coarse form of fibrillation, which in no way serves to fix the joint. It also occurs in muscles which do not move the joint in question; thus in Case 6, moving the knee produced spasm in the anterior crural muscles as well as in the calf, hamstrings, and quadriceps. This form of muscle spasm is clearly different from the guarding shown by the cases with ligamentous or muscular pain; and although its mechanism is at present obscure, it was noticed that in the joints associated with this type of muscle spasm, there was always a complete loss of cartilage as judged by the absence of any joint space in the X-ray.

Radiological findings. Let us now discuss the relation between the clinical groups and the finding of osteoarthritis in the X-ray picture. The cases with a disordered joint mechanism all presented considerable or gross osteoarthritis in the X-ray. In these cases the osteoarthritic changes were often asymmetrical and the symptoms appeared to be related to the degree and nature of the bony and cartilagenous changes present.

On the other hand, the cases of synovitis and ligamentous or muscular strains presented varying degrees of osteoarthritis or none at all (Table I). In these cases the osteoarthritis when present was always symmetrical, occurring equally in the painful and painless knees; and the presence or absence of osteoarthritis had little effect on the clinical picture, this being uniform throughout the groups already described. The incidence of osteoarthritis in this selected group of subjects with painful knees differed little from the incidence of such changes in routine autopsies (2) (4) (8) and it seems probable that the occurrence of pain and osteoarthritis in these cases is little more than a coincidence.

As the symptoms resulting from synovitis or ligamentous and muscular strains were usually relieved by suitable treatment, although any osteoarthritis that was present persisted, it becomes clear that for practical purposes

* This flaccidity may give a false idea of "spasm" because, on contracting the quadriceps, the slack is taken up with a jerk.

we should consider these patients as cases of synovitis or of ligamentous and muscular strains irrespective of any osteoarthritis that may be revealed by the X-rays.

CONCLUSIONS.

(1) In most painful joints of obscure etiology, the source of pain can be demonstrated by local anæsthesia, and on this basis three clinical groups have been described. These consist of cases of ligamentous or muscular "strains," cases of synovitis, and cases with a disordered joint mechanism.

(2) From a study of these clinical groups, it would seem that in joint disease, the relation between pain, muscle spasm, and wasting is not a simple one and requires further study.

(3) The cases with disordered joint mechanism were all associated with considerable or gross osteoarthritic changes, and it seems reasonable to assume that in them the disability results from these changes. On the other hand the finding of slight or moderate osteoarthritis in patients suffering from synovitis, or ligamentous and muscular strains, appears to be little more than a coincidence.

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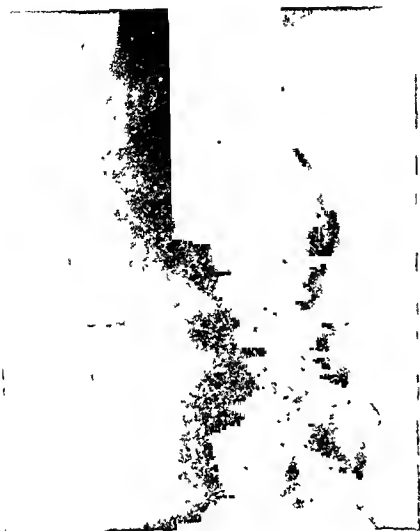


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

Figs. 2 to 9. X-rays of knees showing changes classed as slight osteoarthritis (Fig. 2 and 3), moderato (Fig. 4 and 5), considerable (Fig. 6 and 7), and gross (Fig. 8 and 9).

HYPERTENSION PRODUCED IN THE RABBIT BY PROLONGED RENIN INFUSION.*

By JANET R. HILL and G. W. PICKERING.

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It is now known that hypertension may be produced in the dog by constricting the artery supplying a kidney severed of all nervous connections by transplanting it to the neck (2) or groin (4); when the kidney is removed, the hypertension disappears (2) (5) (11). It is evident therefore that this form of hypertension is due to the release into the circulation of some chemical substance by the kidney. It is known further that in many species, though not in all, a protein like substance, renin, with a powerful and prolonged pressor action, may be extracted from renal cortex (1) (7) (10). There is thus a *prima facie* case for the idea that experimental renal hypertension is due to the ischaemic kidney continually releasing renin into the blood traversing it. There is no need to discuss here all the evidence relevant to this hypothesis, which has received no direct proof. But there is one formidable difficulty in the way of its acceptance, namely, the doubt as to whether renin is capable of producing a long maintained hypertension. This doubt is occasioned by the observation, first made by Tigerstedt and Bergman (10) and repeatedly confirmed by others (1), that in an anaesthetised animal repeated injections of renin give diminishing responses which may ultimately be extinguished. In a previous paper it was shown that in the unanaesthetised rabbit repeated injections of a given dose of renin give fairly constant responses provided that sufficient time is allowed between injections for the arterial pressure to return to normal; it was also shown that the response to renin is reduced by anaesthesia (7). Nevertheless the doubt remains, and it is the first object of this paper to show that with suitable dosage it is possible to produce maintained hypertension by the continuous intravenous infusion of renin.

* Work done on behalf of the Medical Research Council.

Method.

Four male and a female adult rabbits fed on a mixed diet were used ; no food or drink was given during the experiments or for the previous 12 hours. The left ear received the infusion and had been rendered insensitive by previous aseptic section of the two chief sensory nerves at the base of the ear ; the arterial pressure was measured on the right ear by Grant and Rothschild's capsular method, precautions being taken, by adequate covering of the animal and environmental temperature, to keep the ears flushed. Before the infusion the animals responses to intravenous injection of 4 mg. tyramine and, 15 min. later, to 1 mg. renin were tested ; an hour after the renin injection the urine was collected from the male animals for half an hour, by catheterising the bladder, to determine the basal rate of secretion. A specimen of heparinised blood having been drawn from an ear vein, the animal was placed in a wooden rabbit box without top and with a waxed bottom perforated in one place to collect urine naturally voided ; the body of the animal was covered by a towel over which a rubber hot water bottle was placed ; the head remained free and nothing pressed on the neck. Under such conditions the animal would remain quiet for many hours. After a satisfactory base line for the arterial pressure had been obtained, infusion was begun.

The infusion was delivered from two burettes connected by a glass Y tube and narrow rubber tubing to a small needle inserted into a marginal vein of the left ear, and fixed there by means of bull dog clips, the jaws of which were protected by rubber. A semi-vertical and partially fixed position of the ear was obtained by passing through its tip a thread which was drawn up and over a smooth bar and counter-weighted. To secure steady rates of infusion at rates as slow as 1 c.c. per 10 min. the device for an oil gravity feed described by Burn and Dale (3) was used. The two burettes, containing respectively a filtered solution of renin in 0.9% saline and 0.9% saline alone, were fixed vertically above the ear. The free space at the top of the burettes was filled with mineral oil faintly coloured with Scharlach Red, and the open ends of the burettes were fitted with rubber bungs perforated to carry glass capillary tubes of 1 mm. bore and 5 cm. long. These capillary tubes were connected by rubber tubing and a Y tube to an adjustable reservoir, the whole being filled with mineral oil, which thus presented a continuous column between the upper surface of the solution in the burette and the reservoir. The infusion was begun from the burette containing saline alone and, after an appropriate rate had been secured, was continued with the renin solution by rearrangement of clips. About 20 min. after the infusion had been stopped, the animals were catheterised, and a second specimen of blood was obtained ; measurements of arterial pressure were continued for an hour or more after stopping the infusion or until the arterial pressure had returned to normal ; the responses to injections of 4 mg. tyramine and, 15 min. later, 1 mg. renin were then again tested. The

time elapsing between the end of the infusion and the testing of these responses is shown in column 7 of Table I.

The renin used in these experiments was prepared from fresh rabbit's kidney by a method previously described (9). Briefly, a saline extract of alcohol dried kidney was half saturated with ammonium sulphate; after 24 hours in the cold, the precipitate was separated by filtration and dialysed against tap water for 24 hours; the dialyser contents were centrifuged and the supernatant solution evaporated to dryness in a vacuum desiccator. Two powders so prepared were used. They had activities of 0.5 and 0.6 units per mg., 1 unit being the renin content of 100 mg. of our standard kidney powder (7). Stored at 3°C., the powders maintained their activity unchanged for several months. Solutions of 0.2 and 2.0 mg. per c.c. in 0.9% sodium chloride solution were prepared for infusion on the day of experiment.

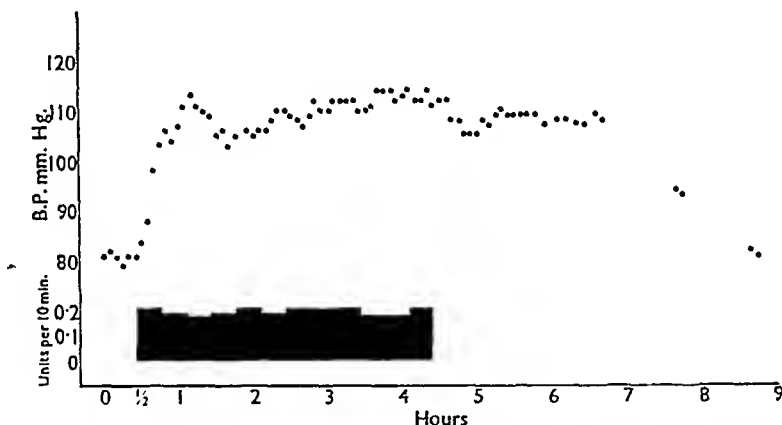


Fig. 1. July the 11th, 1939. Male rabbit, 9, weight about 3.0 kg.. Chart shows arterial pressure measured by capsulo method from central artery of flushed right ear. Infusion delivered into vein of left ear at a rate of approximately 1.5 c.c. per 10 min., saline being run in from 10th to 25th minute, and then a solution of renin, 0.2 mg. per c.c. of 0.9% sodium chloride. The rate of infusion of renin in 10 min. periods is charted as units per 10 min..

Results.

The results obtained in these experiments were dependent chiefly on the rate at which renin was infused.

Infusions at slow rates. In 5 rabbits a solution containing 0.2 mg. renin per c.c. was infused at such a rate that the animal received 0.16 to 0.21 units of renin per 10 minutes, the infusion being maintained at this rate for 4 hours. The experiments are summarised in the first section of Table I and are illustrated by Fig. 1. In each experiment the arterial pressure began to rise a few minutes after the infusion was started and gradually reached a maximum value 22 to 34 mm. Hg. above the initial level. Some idea of the form of the blood pressure curve may be obtained by noting that, in the 5 experiments, the average time at which the arterial pressure had risen to

TABLE I.

Rabbit.	Renin infused, units per 10 min.	Duration, infusion, min.	Arterial pressure, mm. Hg.				Response to 1 mg. renin, mm. Hg.		Response to 4 mg. tyramine, mm. Hg.		Net loss or gain during infusion	
			initial	maxi- mum	end infusion	last reading (min. after end infusion)	before	after	before	after	Water c.c.	NaCl. g.
6	0.21	244	82	104	100	82 at 43 min.	—	—	—	—	+12	+0.1
8	0.19	245	84	112	110	90 at 260 "	—	—	—	—	—39	—0.4
9	0.21	249	80	114	112	82 at 240 "	—	—	—	—	—	—
118	0.19	240	82	106	100	80 at 110 "	18	18	—	—	+50	+0.4
184	0.16	235	56	86	84	57 at 163 "	—	—	—	—	—	—
6	1.7	130	86	120	101	86 at 31 "	19	11	34	25	—67	—0.55
8	1.7	150	90	130	104	98 at 60 "	37	10	—	—	—61	—0.6
8	1.6	136	82	128	100	92 at 60 "	21	7	33	24	—40	—0.4
8	1.3	80	82	119	89	82 at 20 "	27	16	33	19	—64	—0.5
9	1.3	122	90	120	100	102 at 60 "	—	—	—	—	—33	—0.4
9	1.6	126	86	134	104	92 at 60 "	31	8	—	—	—73	—0.1
6	1.5	106	70	104	90	82 at 109 "	10	11	46	33	+39	+0.2
9	1.6	91	82	116	101	82 at 94 "	10	16	38	30	+52	+0.5
118	1.2	100	73	104	103	75 at 140 "	16	17	49	52	+10	+0.1

within 5 mm. Hg. of its peak value was 35 min., and the average time for attainment of the peak was 120 min., after beginning the infusion. The blood pressure remained within 5 mm. Hg. of its peak value until the infusion was terminated; no tendency for the arterial pressure to fall during the infusion was observed in any animal. After stopping the infusion the pressure remained at its plateau level for some minutes and then slowly fell, the resting level being regained at a time varying from 43 to over 260 min. in the various experiments. The average duration of the hypertension, maintained within 5 mm. Hg. of its peak level, was about 4 hrs in these experiments.

Infusions at fast rates. Our first experiments were made with solutions containing 2.0 mg. renin per c.c. and infused at rates of between 1.2 and 1.7 units per 10 min.. With such rates the arterial pressure rose quickly, reaching a maximum value 34 to 46 mm. Hg. above the initial level in a space of between 6 and 20 min.. This maximum value, however, was not

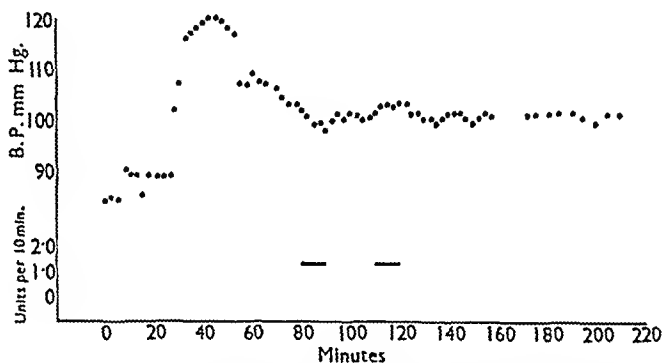


Fig. 2. May the 22nd, 1939. Rabbit as in Fig. 1. The renin solution in this experiment contained 2 mg. per c.c. of 0.9% sodium chloride, and was infused at a rate of about 1.1 c.c. per 10 min.. In comparison with previous figure it should be noted that the rate of delivery of renin is plotted on a smaller, and the time on a larger, scale.

maintained for more than about 20 min., the arterial pressure then falling away in spite of the maintenance of the infusion, until finally, when the infusion was terminated 80 to 150 min. after its inception, the arterial pressure had fallen to within 7 to 18 mm. of its initial value. After stopping the infusion the arterial pressure fell towards normal at a variable rate. These experiments are summarised in the second section of the table and illustrated by Fig. 2.

The cause of the failure to maintain hypertension with high rates of renin infusion. At the end of these experiments in which renin had been infused at high rates there was evidence of profound disturbance of the animal. Thus the rabbits seemed exhausted; after being removed from their boxes they lay quiescent on the floor whereas after experiments with slower rates they would hop about and eat in normal fashion. Again, reduced responses

were observed both to renin and to tyramine, when the blood pressure had returned to normal or an hour after stopping the infusion.

This disturbance was not due to the animals being overheated. Precautions were taken to avoid this, and rectal temperature measured before and again after the infusions showed no constant or significant rise.

In a previous paper (9) it has been shown that renin prepared in the way described causes the unanæsthetised rabbit to lose large quantities of water and sodium chloride in the urine. In view of the large urinary losses of sodium chloride which occur in adrenalectomised dogs and of the failure of experimental renal hypertension to be maintained in such animals (2) (5), it appeared to us particularly important to ascertain whether the phenomenon now under discussion was due to salt lack. It was with this end in view that the measures described for urine collection during these experiments were devised. The total loss or gain of water and sodium chloride induced

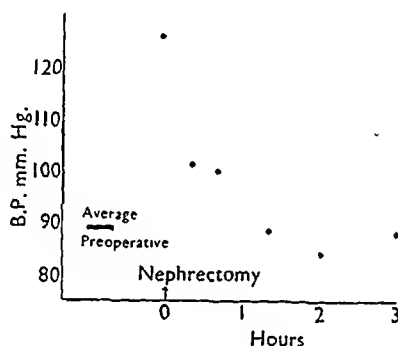


Fig. 3. Female rabbit 185. Right kidney removed 17.10.38, left renal artery constricted and kidney brought out under skin of loin 31.10.38. The arterial pressure rose from a pre-operative average of 89 mm. Hg. to 103 on 1.11.38 and to 126 on 8.11.38, on which day the kidney was removed under ether anaesthesia lasting 15 min.. The subsequent fall of arterial pressure is shown.

by the renin infusion was computed by subtracting from the urinary loss of these constituents during the infusion the sum of amount of water and salt given intravenously and the urinary excretion which would have occurred during the time occupied by the infusion had no renin been given; the latter calculated from the rate of excretion measured before infusion was always small.

From this point of view our experiments fall into 3 chief groups and are so arranged in Table I. The first group consists of five experiments in which dilute solutions containing 0.2 mg. renin were infused at rates corresponding to about 0.2 units per 10 min.. The amounts of water and salt lost in the urine were less in two and greater in one experiment than the amounts infused intravenously; in two experiments the amounts were not measured. The arterial blood pressure was maintained in all these experiments, irrespective of whether there was a loss or gain of salt and water.

The second paragraph in Table I summarises 6 experiments in which solutions containing 2.0 mg. renin per c.e. were infused at rates corresponding to about 1.5 units per 10 min.; and in these the arterial pressure was not maintained. In the first five experiments it may be seen that the loss of water varied between 33 and 67 c.e. and of sodium chloride between 0.4 and 0.6 g.. In the sixth experiment 5 c.e. of 10% sodium chloride solution was injected intravenously 95 min. after beginning the infusion, at a time when the arterial pressure had fallen to 104 from its peak value of 134 mm. Hg.. The injection was followed within 2 min. by a rise of arterial pressure to 117 mm., but the rise was not maintained, and 18 min. after the injection the pressure had returned to 105 mm. Hg.. The net loss of sodium chloride during the infusion was in this way reduced to 0.1 g., but there still remained a water loss of 73 c.e..

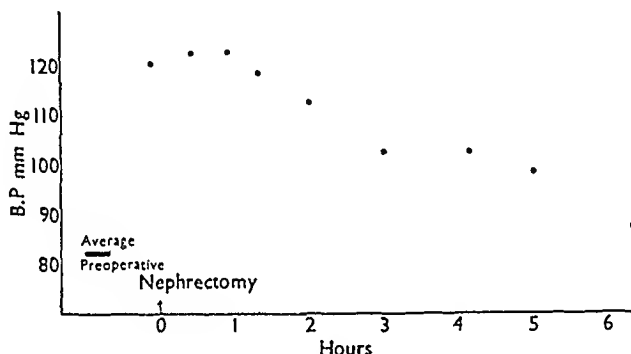


Fig. 4. Female rabbit 244. Right kidney removed 31.7.39, left renal artery constricted 14.8.39, and left kidney removed 18.8.39. The arterial pressure rose from a pre-operative average of 82 to 102 mm. Hg. 5 hours after constricting the left renal artery, and to 120 mm. Hg just before extirpating the left kidney. The operation for left nephrectomy occupied 8 min., and the duration of anaesthesia was about 15 min.. The fall of arterial pressure following left reflectory is shown.

It was shown in a previous paper (9) that the sodium and chloride contents of the urine secreted in response to renin approximate closely to those of the plasma. It was not expected therefore, that the large losses of chloride in these experiments would be associated with any change in plasma chloride, nor was any change found in the three experiments where it was looked for. Some hæmo-concentration was expected, but in these experiments this, estimated by the hæmoglobin content, amounted to under 8%. It seems probable therefore that most of the water and chloride lost in response to renin is of extravascular origin.

In order finally to exclude loss of water and salt as a factor in the failure of the arterial pressure to maintain itself at its highest level, we made experiments in which more dilute solutions of renin, 0.25 to 0.3 mg. per c.e. in saline, were infused at rates of 1.2 to 1.6 units per 10 min., the object being to give intravenously in the infusion at least as much water and salt

as was lost in the urine. The three experiments so made are summarised in the third paragraph in Table I, from which it may be seen that the amounts of water and salt infused intravenously were in definite and often pronounced excess of the urinary loss, so that a net gain of both constituents resulted. Nevertheless the arterial pressure was not maintained in two out of the three experiments; in the third animal the arterial pressure was maintained but in this instance no comparable experiment involving water and salt loss was made. It may be noted in passing that the responses to tyramine and renin showed no definite reduction after the infusion in these experiments. Reference to the table shows, however, that the responses were tested at a much later time, 94 to 140 min. after the infusion, than they were in the preceding group. This difference between the two groups of experiments may have no other significance.

We may therefore conclude from these experiments that losses of salt and water are not the chief causes of the failure for the arterial pressure to be maintained with high rates of renin infusion, though they may be contributory. The falling away of the arterial pressure seems to be a manifestation of over-dosage and presumably represents a toxic effect of large quantities of renin or some substance associated with it in our preparation. It is presumably another aspect of the phenomenon so frequently observed in anæsthetised animals, namely diminishing responses to successive injections. In a previous paper (7) we have pointed out that saline extracts of alcohol dried rabbit's kidney give a preliminary depressor response in the urethanised rabbit, and that this response is due to two substances, one differing from renin in its stability to heat and greater adsorbability on charcoal and one resembling renin in its behaviour to these and other measures and possibly identical with it. To remove this first substance, we treated our renin solution (2 mg. per c.c.) with 1 mg. Norit per c.c., subsequently filtering and assaying the solution before infusing it. This experiment was the fifth in the second paragraph in the table and it may be seen that its result differed in no way from the others. However, until renin has been obtained in a pure form we cannot exclude the possibility that the phenomenon now being discussed results from the cumulative effect of some impurity. Alternatively it may be due directly to over-dosage with renin itself. A similar and imperfectly explained phenomenon, tachyphylaxis, is known to occur with posterior pituitary extract.

Discussion.

If the hypertension resulting from constriction of the renal arteries is due to the maintained release of renin into the blood flowing through the ischæmic kidney, then a hypertension identical in its main features should result from continued intravenous infusion of renin. The experiments described in this paper have shown that, provided excessive dosage is not used, it is in fact possible to maintain hypertension by the continued infusion

of renin, at least for a period of over 5 hours; and inspection of Fig. 1 provides no reason for doubting that, by prolonging the infusion, hypertension might have been maintained indefinitely. The failure of larger doses to maintain hypertension is of little importance in this connection, for they were probably unphysiological.

We have looked for, but not observed any points of difference between experimental renal and renin hypertension. Two points of similarity are striking enough to be mentioned. As we have previously pointed out (8), the appearance of the ear vessels, when the animal is warm, is not significantly altered by the onset of hypertension due to renal artery constriction; nor is their appearance to the naked eye altered during the intravenous infusion of renin in the doses here used. This behaviour of the ear vessels to renin, as Landis, Montgomery and Sparkman (6) have pointed out, contrasts sharply with the behaviour to other known vasoconstrictor substances.

But the most significant point of similarity which we have established between the two hypertensions is the duration of hypertension after removing the agent's source of supply. Table II summarises the findings in 5 rabbits

TABLE II.

Summarises the rate of disappearance of hypertension after removing the ischæmic kidney; in the first 2 rabbits on the 4th day, and in the last 3 rabbits on the 6th day, after constricting the renal artery. In all these rabbits the right kidney had been removed a fortnight before constricting the left renal artery.

Rabbit.	Arterial pressure mm. Hg..			Duration hypertension after L. nephrectomy. (hrs and min.).
	before and 5 to 12 hrs after constricting L. renal artery.		before L. nephrectomy.	
244	82	102	120	6.30
246	81	115	120	> 6.0 < 24.0
200	81	96	96	2.30
201	80	90	90	0.45
185	80	103	123	1.45

in which, two weeks after removing the right kidney, hypertension was produced by constricting the left renal artery; 4 to 6 days later the left kidney was removed under light ether anæsthesia, the operation taking 5 to 10 min. and the anæsthesia lasting altogether 10 to 20 min.. It may be seen that the degree of hypertension attained in these animals was similar to that produced by intravenous infusion of the smaller dose of renin, being in the animals with renal ischæmia 10 to 39 mm. Hg. above the pre-operative level. After removing the ischæmic kidney, the arterial pressure regained its pre-operative level in a time which varied from 40 min. with the least hypertension to about 6½ hours with the greatest. Comparison with

Table I shows that these times are very similar to the time taken for the arterial pressure to regain its normal value when intravenous infusion of renin is stopped. Comparison of Fig. 1 with Figs 3 and 4, taken from the experiments on the animals with renal ischaemia, emphasises the similarity.

It had been our intention to carry these experiments farther in comparing other features of the two hypertensions, but circumstances now render completion of this programme unlikely. Enough has been done, however, to show that a maintained hypertension not dissimilar to experimental renal hypertension may be produced by the continued addition of renin to the circulation.

SUMMARY.

1. Solutions of renin in 0.9% saline infused intravenously for 4 hours into unanaesthetised rabbits at rates corresponding to about 0.2 units renin per 10 min. produce a pronounced rise of blood pressure which shows no tendency to decline while the infusion is continued. After stopping the infusion the blood pressure slowly falls, reaching its normal level in about 1 to 4 hours.

2. Intravenous infusion of renin at rates corresponding to about 1.5 units per 10 min., produces a rapid and large rise of blood pressure maintained for about 20 min. and then falling towards the normal level while the rate of infusion is continued.

3. The falling off of the hypertension during infusion of renin at high rates is not due to the loss of salt and water in the urine which large doses of renin produce. It is presumably due to the cumulative effect on the cardiovascular system either of excessive amounts of renin itself or of some impurity present in our preparations.

4. Renin hypertension resembles experimental renal hypertension both in the macroscopic appearance of the ear vessels and in the rate at which the hypertension disappears when supply of the agent is stopped.

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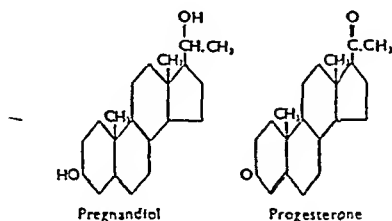
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THE EXCRETION OF PREGNANDIOL AND THE CORPUS LUTEUM.

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THAT pregnandiol is excreted in considerable quantity in the urine of pregnant women has been known for nearly ten years. It was one of the earliest of the sterol compounds of the sex hormone group to be isolated from such urines (16). But this compound proved to be entirely inactive physiologically and probably on this account received scanty attention from physiologists and clinicians for some years. It early attracted great interest from sterol chemists, however, and was carefully studied by Butenandt (5) in particular. Butenandt was able to elucidate the structural formula of pregnandiol, and used this knowledge to establish the chemical formula of progesterone, which proved to be very similar. Proceeding from this point Butenandt and Schmidt (6) were soon able to convert pregnandiol by chemical means into progesterone. This transformation not only emphasized the close chemical relation between the two compounds and confirmed the structural formula of progesterone, but for the first time made possible the chemical preparation of the latter substance.

The chemical formula of pregnandiol and progesterone as established by Butenandt are shown below :—



* Our grateful thanks are due to Professor Chassar Moir for granting free access to his clinical material, to Mr. J. A. Stallworthy for much diagnostic help and for the endometrial biopsies, to Dr. A. H. T. Robb Smith for pathological reports on these biopsies, to the Nursing Staff of the Radcliffe Infirmary for that close co-operation which was so essential to the satisfactory collection of complete daily urine specimens over long periods, and to Messrs. Organon for generous supplies of Progesterone.

It will be seen that the two compounds differ essentially in that the two ketonic groups of progesterone have been reduced to hydroxyl groups in the pregnandiol molecule. It was because of this close similarity in structure that Butenandt early suspected that pregnandiol was in fact an excretory product of corpus luteum metabolism, but little confirmatory evidence of such a hypothesis could be collected because of the lack of satisfactory methods for the detection of small amounts of pregnandiol in the urine.

A step forward was made, however, when it was shown in 1936 by Odell and Marrian (17) that only a relatively small part of the pregnandiol of urine is normally present in the free state, the greater part being in a combined form from which it can be freed by acid hydrolysis. In the same year Venning and Browne (23) reported the isolation from pregnancy urine of considerable quantities of a crystalline salt which on analysis proved to be a mono-sodium salt of pregnandiol glucuronic acid. The quantities of this compound which they were able to extract represented amounts of pregnandiol considerably in excess of any previously reported to have been obtained from such urines.

A year later Venning (21) published details of a method for the estimation of this pregnandiol complex based on its extraction in relatively pure form from urine, and the direct weighing of the acetone insoluble precipitate so obtained.

By using this method Venning and Browne have been able to study quantitatively the excretion of pregnandiol in the urine under a variety of conditions. The results of these investigations all tended to support the hypothesis of Butenandt that pregnandiol is an excretory product of corpus luteum metabolism. Thus it was shown that pregnandiol appears early in the urine in pregnancy and continues to be excreted in increasing amounts up to full term, when it speedily falls to zero within two or three days.

Largely because their method of pregnandiol extraction gave much higher yields than earlier methods and was therefore much more sensitive, Venning and Browne (24) were able to obtain clear evidence that pregnandiol is excreted in definite though much smaller amounts in the urine during the normal menstrual cycle. In a series of cases studied, pregnandiol appeared in all only during the second half of the menstrual cycle when, as is well known, the corpus luteum is developing, and fell again to zero one to two days before the onset of bleeding. In selected cases in which the exact time of ovulation could be dated by the experiencing of the so-called "mittelschmerz," it was possible to show that pregnandiol appeared in the urine one to two days after this event. These observations provided therefore, strong clinical evidence that Butenandt's view of the significance of pregnandiol was indeed the correct one.

As further evidence Venning and Browne (24) studied the effects on pregnandiol excretion of the injection of progesterone. Quantities of from 19 to 30 mg. of progesterone were injected into women in whom there was

reasonable certainty that no corpus luteum was present at the time of injection. From 12 to 46 per cent. of the injected progesterone was recovered in the form of pregnandiol glucuronide in the urine. Thus it would appear not only that pregnandiol is an excretory product of progesterone metabolism but that it is a major one. But recent attempts to confirm this latter claim, to be more fully considered later, have been unable to confirm that pregnandiol appears in the urine after progesterone injection. Nevertheless the findings of Venning and Browne, with their far reaching implications and with the wide scope they offer for more detailed study of corpus luteum metabolism, amply justify careful confirmation and extension in the clinical as well as the physiological field.

In the work presented in this paper we have attempted to confirm some of the claims of Venning and Browne and in addition, to study such suitable clinical cases as have become available for investigation, with particular reference to their thesis that pregnandiol glucuronide excretion provides an index of the intensity of corpus luteum activity.

Estimation of pregnandiol.

Several alternative methods have already been suggested for the detection of pregnandiol in urine, but of these the method of Venning (21) is the only one which lays any claim to quantitative accuracy.

Weil (26) has made use of the slight solubility of free pregnandiol in water, and allows the urine to stand in an incubator at 37° until bacterial decomposition has hydrolysed the soluble pregnandiol glucuronide into the insoluble free state. This method was originally designed for pregnant mare's urine, but in a few preliminary trials of the method on human pregnancy urine we have been unable to satisfy ourselves of its suitability for routine purposes, nor have we been able to obtain evidence in this way of the presence of pregnandiol in urine samples already known to contain small quantities.

An alternative method of hydrolysis has been proposed by Veitsch, Milone and Everitt (20) using the enzyme emulsin which yields up to 50 mg. per litre of pregnandiol from pregnancy urine, but we have had no experience with this method.

Beall (2) has proposed the preliminary extraction of both free and combined forms by absorption on benzoic acid, and their subsequent hydrolysis by boiling with acid. The pregnandiol is obtained in crude form as a dry residue and is purified by crystallization. We have made a number of trials with this method and have compared the results with those given by the Venning method previously mentioned. Comparison of these two methods is in some respects unjustified because that of Beall includes both free and combined forms of pregnandiol whereas the method of Venning estimates only that present as glucuronide. It was nevertheless of interest because it offered prospects of determining by difference the proportion of

free pregnandiol. There appears to be a fair correspondence between the results of the two methods when these are based on the weights of crude extracts, but as recrystallization of the free pregnandiol is a less quantitative procedure than is reprecipitation of the glucuronide by acetone we have not been able to obtain by Beall's method results comparable to those of the second precipitation in the Venning method.

Typical comparative results by the two methods are given below, all being expressed as mg. of actual pregnandiol per specimen.

	<i>Venning method.</i>		<i>Beall method.</i>	
	1st Pp.	2nd Pp.	Dry weight.	Recryst.
(1)	39.5	33.0	30.2	19.0
(2)	31.4	22.0	31.2	13.0
(3)	53.2	16.2	57.6	18.2
(4)	37.6	29.2	43.2	19.5
(5)	31.1	23.0	21.7	12.0
(6)	39.2	22.5	47.1	17.3
<i>Mean</i>	38.7	24.3	38.5	16.5

The relatively close correspondence between the first precipitation by the Venning method and the dry weight of the residue obtained by the Beall method suggested that the latter might be made to yield results comparable to the former, if only more quantitative means could be found for purifying the free pregnandiol in the dry residue. This, however, proved not to be the case, for further investigation showed that the urine residues after the extraction with benzoic acid in the Beall method still contained further pregnandiol glucuronide which could be extracted with butyl alcohol. Since this residual pregnandiol glucuronide, amounting to some 15 per cent. of the total, showed that the absorption on benzoic acid was incomplete, and since we were unable to improve it by change in the pH at which absorption occurred, the method of Beall was abandoned, and that of Venning used exclusively in our work.

Experience of many hundred estimations by this method has led to the adoption of only minor modifications in technique. Some of these have since been published independently by Venning (22) herself and need not be repeated here. Her method consists essentially in extracting the urine several times with butyl alcohol, treating the extract with caustic soda, and precipitating pregnandiol glucuronide in crude form by acetone. This crude product, which will be called the first acetone precipitate, is dissolved in water and reprecipitated with acetone as the second acetone precipitate which is weighed.

In carrying out the first extraction of the urine with butyl alcohol it has been found advantageous first to saturate the urine with sodium chloride at room temperature. By diminishing the solubility of the butyl alcohol in the aqueous phase this has several advantages. It ensures an increased recovery of the butyl alcohol layer after the first extraction with a resultant more efficient removal of the extractives from the aqueous phase. Furthermore such saturation appears to result in a more rapid separation of the shaken mixture into two layers on standing. Previous to the use of this sodium chloride saturation of the urine, some of the butyl alcohol phase frequently tended to remain in fine suspension. Because of this each extraction, after shaking, was left, as a routine, for several hours to separate, the total period of extraction with the four portions of butyl alcohol being spread over 24 hours. After saturating the urine with sodium chloride this time could be shortened considerably.

In urines containing an appreciable quantity of albumen voluminous precipitates are usually formed on shaking with butyl alcohol, and these can only be partially resolved by long centrifuging. These paste like emulsions of protein, urine and butyl alcohol are best broken up by adding further butyl alcohol, shaking well and again centrifuging. By repeating this process two or three times the whole butyl alcohol phase can be recovered leaving a firm pack of protein at the bottom of the tube.

In the first precipitation with acetone at 95 per cent. concentration the deposit from non-pregnant urines is usually finely granular and amorphous, but occasionally it has a fat-like appearance and adheres to the bottom and sides of the flask. It is our belief that this second type of precipitate is not true pregnandiol glucuronide and the greater part of it usually fails to reprecipitate during the second acetone precipitation. We have always weighed the first as well as the second acetone precipitate and find only a rough relation between them. This relationship is shown graphically in the Fig. 1 plotted from fifty results taken at random. The second precipitate tends to attain at its maximum 85 per cent. of the weight of the first, but many samples fall far short of this, so that it is quite unsafe to rely on the weight of the first precipitate as an index of the pregnandiol glucuronide concentration. It has been our impression that samples showing a poor yield in the second precipitation tend to have low melting points. Observations on a urine which showed a gross discrepancy between the weights of the first and second precipitates are reported later in the section headed "atypical excretion products." The values obtained for this atypical urine are not included among the points plotted in Fig. 1.

In the second precipitation true pregnandiol glucuronide always appears as a white flocculent precipitate or else as a visibly crystalline one, and judging by melting points the latter is not necessarily purer than the former. Other types of precipitate at this stage, such for instance as a milky opalescence which settles very slowly over a period of days, should be regarded

with suspicion as they probably do not contain pregnandiol glucuronide in high percentage.

In making our calculations we have corrected for the incomplete yield by making use of the percentage recoveries found by Venning (21) for different known concentrations in urine. In trial recoveries of known amounts of pregnandiol glucuronide added to portions of a urine giving a very low blank value, we have obtained yields only slightly lower than did Venning.

All our results have been expressed as mg. pregnandiol excreted per 24 hours. This implies a complete collection of the 24 hour specimen of urine, and this we believe to have been achieved in the great majority of specimens. Occasionally part of a specimen has been lost, but to avoid errors due to this cause we have made it a uniform practice to estimate also the daily excretion of creatine + creatinine by the method of Folin (8). By doing this a fairly constant figure for the daily excretion is obtained, and any 24 hour specimen of urine which is incomplete is easily detected, this being reflected in a big drop in the apparent excretion of creatine + creatinine for that day. Many women, especially those likely to be excreting pregnandiol, excrete creatine in the urine also. It is because of this that we have estimated daily creatine + creatinine rather than the creatinine alone for Shaffer (18) has shown that the sum of the two is likely to be the more constant in output. It would seem highly desirable to adopt this method of checking the completeness of 24 hour specimens in all hormonal work in which results are expressed in terms of 24 hourly excretion.

The normal menstrual cycle.

Venning and Browne (24) have reported fully on the study of ten menstrual cycles in normal women. They found pregnandiol in the urine only after ovulation had occurred, mainly during the luteal phase. The total quantity excreted in the urine during the whole of this phase varied widely in different individuals without evident cause, the extreme limits being 3 mg. to 54 mg. per cycle. In the majority of cases the curve of excretion tended to rise to a maximum about one week before the next bleeding was due, and then to fall gradually, always reaching zero before bleeding actually occurred. Thus, although there were wide variations in the total quantity of pregnandiol excreted, the curve of excretion had in all cases essentially the same time relation to menstruation.

Similar observations have been made on normal menstrual cycles by Wilson, Randall and Osterberg (28) and also by Stover and Pratt (19) and the results of both groups of workers are in good agreement with those of Venning and Browne.

We have studied five normal menstrual cycles in women who were detained in hospital for reasons unlikely to disturb this rhythm, and who gave an entirely normal menstrual history. The results obtained in this series are shown in Figs 2 to 6. It will be seen that these results also agree

with those of the previously mentioned workers, both in range of quantitative variation and also in their time relations to the menstrual bleeding. In none was any trace of pregnandiol observed during the first or pre-ovulatory half of the cycle. The total quantity of pregnandiol excreted during the whole luteal phase showed wide variation, even in this small series and varied from 69 mg. down to 3 mg. of pregnandiol per cycle. We did not observe any clear clinical difference between those with low and those with high excretion curves, though it is possible that the latter tend to be more vigorous in type.

The mean curve of excretion is shown in Fig. 7, and agrees well with similar curves of other workers, which for comparative purposes are indicated in the same figure.

These results fully support the claim of Venning and Browne that pregnandiol appears in the urine of the normal and non-pregnant woman only during the luteal phase of the menstrual cycle.

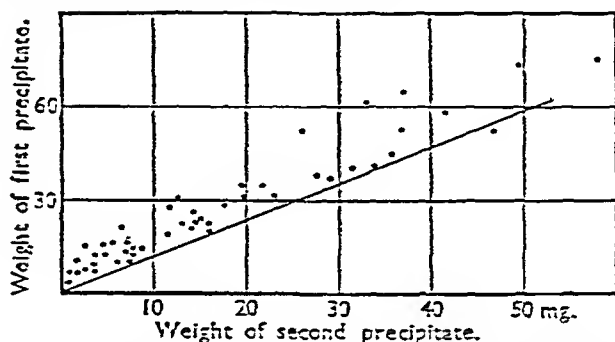
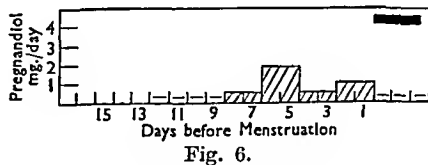
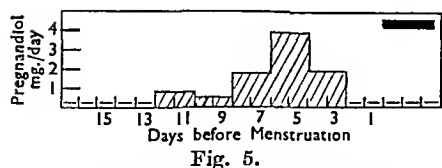
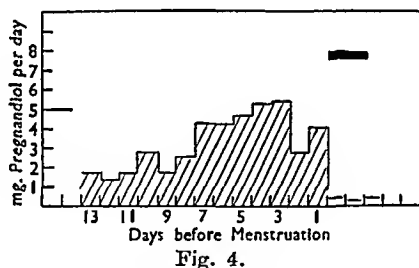
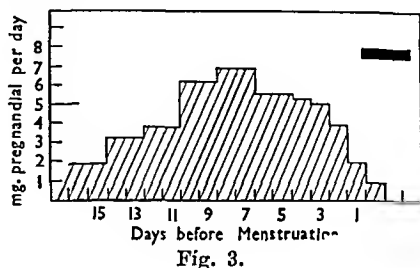
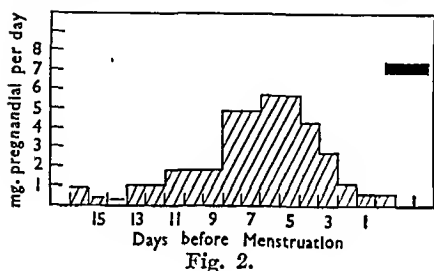


Fig. 1. The relation between the weights of the first and second acetone precipitates in the Venning (21) method of pregnandiol estimation showing lack of a close relation between the two.

It is clear from these curves that isolated pregnandiol estimations carried out on specimens of urine obtained on only one or two days of the menstrual cycle are very likely to give an erroneous impression of the actual pregnandiol excretion curve. The construction of a complete curve is too laborious for general clinical use even if it is confined to the second half of the menstrual cycle. For the purpose of determining rapidly if pregnandiol is being excreted during the luteal phase, an analysis of the urine on the fifth and sixth days preceding a menstrual bleeding is most likely to afford the information required. In cases in which the pregnandiol excretion curve is low, isolated estimations carried out on specimens other than these may show no pregnandiol. It is not justifiable therefore to conclude from such negative estimations that no pregnandiol is being excreted during the luteal phase. For the same reason attempts to correlate the microscopic appearance of the endometrium obtained by biopsy, with pregnandiol excretion, are likely to be of greatly reduced value if the latter is judged by isolated pregnandiol estimations and not by the complete curve.

Hamblen, Ashley and Baptist (10) have obtained results indicating a very irregular excretion curve of pregnandiol during the luteal phase, with days on which no pregnandiol is excreted. They point out that the excretion must depend on renal activity and imply that in this way can be explained the temporary interruption of pregnandiol excretion. The irregularity of their curves contrasts with our own and those of other workers, which tend to follow a much smoother course, and they would therefore appear to require confirmation. That pregnandiol excretion must depend on the degree of renal activity may be admitted, but that it can in this way be interrupted for periods as long as 24 hours is contrary to experience of all



Figs. 2 to 6. Pregndiol excretion during the luteal phase of normal menstrual cycles. Black rectangle marks menstrual bleeding.

other excretory products. More direct evidence that pregnandiol excretion can be influenced for short periods by varying renal function is presented below.

Hamblen, Ashley and Baptist also claim that the excretion of pregnandiol may continue during the actual menstrual flow. Although it must be admitted that the presence of blood in the urine complicates the estimation and contaminates the final precipitate, yet we found no trace of pregnandiol in the urine of any of our subjects after bleeding has commenced, and the experience of Venning and Browne (24) and of Wilson, Randall and Osterberg (28) would appear to be similar.

The influence of renal function.

The most convenient circumstances in which to observe the effects of renal efficiency on pregnandiol excretion in a short experiment are during actual labour itself, for at that time the excretion of pregnandiol has attained its maximum level and the events associated with the passage of the foetus through the birth canal result apparently in a temporary impairment of renal function lasting a few hours. It is true of course that this temporary interruption of renal adequacy, which possibly results from back pressure in the renal pelvis due to pressure of the foetus on the ureters, is not strictly to be compared with the spontaneous variations in renal activity which are known to occur throughout the normal day. But the changes in pregnandiol excretion which occur at this time do illustrate clearly the dependence of pregnandiol for excretion on the renal activity. Comparison of the hourly excretion rates of pregnandiol and of creatine + creatinine during labour was made in two cases and the results are shown in Figs 8 and 9. In these the double line represents pregnandiol excretion and the interrupted line creatine + creatinine. All urine collections were made by catheter.

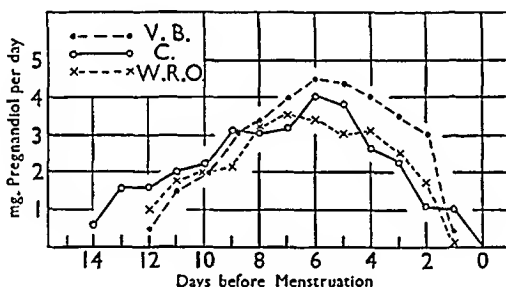


Fig. 7. Mean pregnandiol excretion of five menstrual cycles (C) compared with the similar curves of Venning and Browne (V.B.), and of Wilson, Randall and Osterberg (W.R.O.).

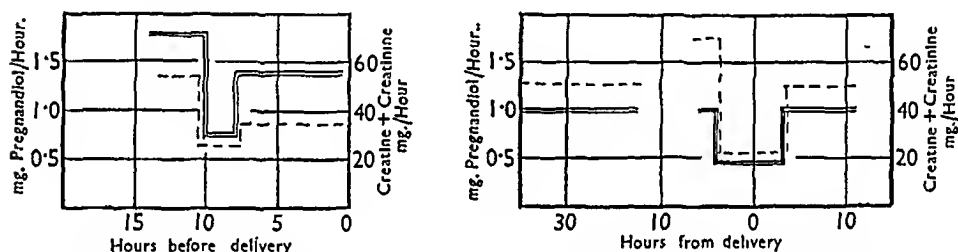
It can be seen that in each a temporary fall occurred in the rate of excretion of creatine + creatinine and that this was accompanied by a roughly equal fall in the pregnandiol excretion. Although simultaneous plasma creatinine determinations were not made, it was felt justifiable to assume that no large or rapid changes in plasma creatinine concentration were likely to occur during this period. On this assumption the changes in hourly creatinine and creatine excretion may be regarded as a reliable rough index of the variations of renal efficiency, for they directly reflect changes in the clearances of these substances by the kidneys, and such clearances are now generally accepted as direct indices of renal activity (7). It would appear therefore that changes in renal activity affect pregnandiol glucuronide excretion to approximately the same extent as they affect the excretion of creatine and creatinine. Since spontaneous variations in renal activity have relatively little effect on the daily creatinine excretion, we find

it difficult to believe that they will have an appreciably greater effect on the excretion of pregnandiol.

Excision of the corpus luteum.

We have been able to follow one case in which the corpus luteum was excised early in pregnancy.

The patient was a married woman, 36 years of age. She had had four previous pregnancies. The menarche had occurred at 17 years and menstruation was usually for five days every 21 to 28 days. It was always associated with dysmennorrhœa. She was admitted to hospital with a history of three months pain in the lower abdomen and of backache. This pain was relieved to some extent by menstruation. There had been severe dyspareunia for three months. The last normal menstrual period had been nine weeks before admission, but there had been a slight show six weeks after this. A cystic mass was found in the mid-line above the symphysis pubis; separate from the slightly enlarged uterus. The Aschheim-Zondek



Figs. 8 and 9. Comparison of pregnandiol excretion with the creatine + creatinine excretion during labour. Double line pregnandiol, interrupted line creatine + creatinine.

test was positive. A left ovarian cyst with recurrent torsion of the pedicle was diagnosed; it was removed, and proved to be a multilocular pseudomucinous cystadenoma of the ovary 10 cm. in diameter with a large corpus luteum. The corpus luteum was typical of pregnancy, a central cystic area being surrounded by a broad zone of true lutein cells and beyond this a narrow and irregular zone of theca lutein cells. Daily injections of 5 mg. of progesterone were started immediately and continued for four days. In spite of this however, lower abdominal pain and vaginal bleeding commenced four days after the removal of the corpus luteum. The external os was partially open. Slight bleeding continued for ten days during which time the contents of the uterus were not completely expelled. On the twentieth day after the removal of the corpus luteum the uterus was emptied. Careful search failed to reveal an ovum.

Daily estimations of urinary pregnandiol began two days after the removal of the corpus luteum, the completeness of urine collections being checked by daily creatine + creatinine estimations. On the second day 7.2 mg. of pregnandiol was recovered, on the next day 4.0 mg., and on the

fifth and sixth days 1.2 mg. and 1.0 mg., respectively. For the next 11 days no trace of pregnandiol was found in the urine. Thus excision of the corpus luteum of pregnancy 67 days after the last menstrual period led to a fall to zero of the pregnandiol excretion in 7 days, and to abortion. The results are shown in Fig. 10. The progesterone injections had evidently no appreciable effect on the pregnandiol excretion.

The prompt disappearance of pregnandiol from the urine in this case provides strong evidence that the corpus luteum of pregnancy is intimately concerned in its production.

Two cases of excision of the corpus luteum during pregnancy in which pregnandiol excretion has been followed have already been reported. In that of Jones and Weil (12) the corpus luteum was removed 58 days after the last menstrual period without abortion occurring. Two days later 15 mg. of pregnandiol were excreted and on the following day 3 mg.. Ten days followed during which no pregnandiol was excreted. On the fifteenth day pregnandiol reappeared and continued to increase in amount until two months later it was 26.4 mg. per day.

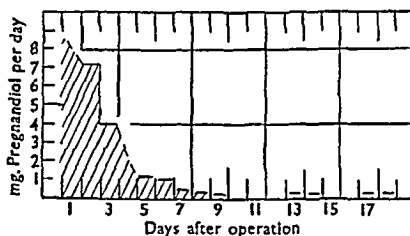


Fig. 10. Effect of excision of corpus luteum on pregnandiol excretion in early pregnancy.

In the case of Browne, Henry and Venning (3) torsion of a cystic ovary occurred and necessitated its removal with the corpus luteum at the fourth month of pregnancy. No details are given but it is stated that pregnandiol excretion fell to a low value for a few days after operation and then rose rapidly again to normal amounts for the rest of a pregnancy which went to full term.

Both these cases differ from our own in that pregnancy was not terminated by the removal of the corpus luteum, and that after a short interval pregnandiol reappeared in the urine. In our own case no opportunity was permitted for such a reappearance since abortion became inevitable.

Browne, Henry and Venning conclude from their case and from other evidence that towards the end of the third month of pregnancy the production of progesterone is taken over by the placenta so that after this time the continued presence of the corpus luteum is no longer necessary for the maintenance of pregnancy. Though the evidence they submit appears to justify their conclusion, there is still much which remains unexplained.

Thus the corpus luteum may be removed even in the first two months without abortion occurring. Ask-Upmark (1), who was one of the first to doubt the accepted views of Fraenkel (9) that the corpus luteum is necessary for continued pregnancy, collected data on 51 cases in all of which the corpus luteum was removed during the first two months. In only 17 of these did abortion occur. Thus it would seem that even at this early date the chorionic villi are capable of taking over the formation of progesterone, though further observations on pregnandiol excretion in such cases are clearly needed before this can be considered firmly established.

In the case of Jones and Weil, which is comparable to our own in that the corpus luteum was removed about two months after the last menstrual period, 8 days elapsed in which pregnandiol was absent from the urine. It seems impossible to explain at present why some early fetuses can and others cannot survive such a period of apparent progesterone deprivation.

The injection of progesterone.

As part of their evidence that urinary pregnandiol is the end product of progesterone metabolism Venning and Browne (24) have claimed that injection of progesterone intramuscularly into women results in the appearance of pregnandiol in the urine. Of 19 and 28 mg. progesterone injected into two patients, in whom there was reasonable certainty that no corpus luteum was present at the time, 46 and 40 per cent. respectively was recovered as sodium pregnandiol glucuronide in the urine; in an oophorectomized woman with an intact uterus 30 mg. was injected and 12 per cent. recovered in the urine. In a short note extending these observations Browne, Henry and Venning (4) state that when progesterone was injected during the luteal phase of the normal menstrual cycle 20 to 30 per cent. was recovered as pregnandiol. They also found that in endometrial hyperplasia the output of pregnandiol was increased by a preliminary treatment with oestrin.

But these findings have not so far been confirmed by other workers. Hamblen, Ashley and Baptist (10) have failed to find any pregnandiol excretion after progesterone injection, and Stover and Pratt who injected 16 mg. in oil of progestin also found no pregnandiol in the urine.

The confirmation of this claim of Venning and Browne is clearly one of such importance that it is unfortunate that all published data on this particular point are scanty. In seeking to recover pregnandiol from the urine after progesterone injection it is clearly undesirable to choose the luteal phase of the cycle, for at this time it is impossible to distinguish between pregnandiol derived from endogenous progesterone and that resulting from the progesterone supplied by injection. To avoid this difficulty we have dealt only with patients in whom we had first found that no endogenous pregnandiol was being produced.

The first case used was one of secondary amenorrhœa associated with mild anorexia nervosa which was rapidly being recovered from. Preliminary

examination of all urine collections over a period of 36 days had already established the absence of pregnandiol, and had shown the limits of variation of the small traces of impurities that come through to the final precipitation and tend to obscure the appearance of traces of pregnandiol. During the second observational period on this patient 10 mg. of progesterone was injected intramuscularly on the first day and 5 mg. on each of the four subsequent days, making a total injection of 30 mg. spread over five days. As a result a slight increase occurred in the weight of the first acetone precipitate but none in the second, and we were unable to convince ourselves that a trace of pregnandiol was excreted, although urine analyses were continued for three days after the cessation of injections.

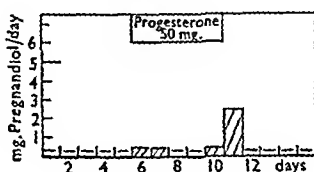


Fig. 11. Pregnandiol excretion following injection of 50 mg. of progesterone.

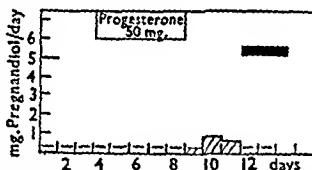


Fig. 12. Pregnandiol excretion after oestrinization of endometrium and subsequent injection of 50 mg. of progesterone. Same case as Fig. 11. Black rectangle marks uterine bleeding.

The second case studied was also one of secondary amenorrhoea, associated with rheumatoid arthritis in an unmarried girl of 23 years. Here also a preliminary examination over 10 days satisfied us that no pregnandiol was being excreted. At the end of this time an endometrial biopsy showed an extremely scanty endometrium containing scattered quiescent acini. Daily injections of 10 mg. of progesterone were begun and continued for five days. A slight rise in the weight of the first acetone precipitate occurred on the first day but no recognizable pregnandiol appeared in the second acetone precipitate until the fifth day when a small trace was present. On the sixth day, however, that is the day following the last injection, another 2.5 mg. was recovered, this appearing as a clearly recognizable crystalline precipitate (Fig. 11). Thus the total weight of pregnandiol actually recovered was 3.0 mg., representing approximately 6 per cent. of the progesterone injected. Such a figure is scarcely comparable to that reported by Venning and Browne who obtained a 12 per cent. recovery in an oophorectomized woman who presumably also had a hypo-

plastic endometrium. It is also a much smaller yield than they obtained in other types of case, but it seemed possible that the relatively poor yield was due to the absence of a normal œstrinized endometrium. To investigate this possibility the same patient was given a course of œstrin, 20,000 units of œstradiol benzoate being injected each day. After three weeks on this treatment a further biopsy of the endometrium showed this to be much more bulky and of non-secretory type, the gland tubules being straight and lined with ciliated epithelium. The gland tubules were not grossly dilated, but the endometrium was still hypoplastic compared with a normal fully œstrinized endometrium. Oestrin injections were then reduced to 4,000 units a day and a fresh series of progesterone injections given, the dosage being as before 10 mg. daily for five days. This resulted once more in the appearance in the urine of pregnandiol, but the total quantity, 1.5 mg., was actually less than was recovered from the same patient after the same dose of progesterone before the œstrin therapy (Fig. 12). The adequacy of the dosage in this case was clearly shown, since the second series of progesterone injections was followed immediately by menstrual bleeding of normal amount and duration, this being the first bleeding that the patient had experienced for six months. Thus the low yield is not due to the hypoplastic endometrium nor is it necessarily increased by a preliminary course of œstrin.

We have also made use of a case of anovular menstruation discussed more fully later, for determining the yield of pregnandiol from injected progesterone. This case appeared particularly suitable for the purpose, for if present views of the ætiology of this condition are correct an ample supply of endogenous œstrin and a well developed endometrium would be present though the latter could not be regarded as truly normal. Here also 10 mg. of progesterone in oil were injected daily intramuscularly for five days after a preliminary observational period of 32 days had failed to show any trace of pregnandiol in the urine. On the fourth and fifth days of injection small but definite quantities of pregnandiol were found in the urine; as a result of the injections 4.5 mg., or 9 per cent. of the amount injected was excreted (Fig. 13). Two days after these injections ended uterine bleeding began. On the second day of this bleeding an endometrial biopsy showed an unusual endometrium, the gland tubules were much convoluted and showed some evidence of secretion, although in places the epithelium was ciliated and true vacuole formation was not present. In some zones there was considerable irregularity in the formation of these tubules. The general effect was one of abnormal luteinization. It is of interest that at a corresponding time in a previous menstrual cycle the endometrium had shown no evidence of secretion (*see page 233*).

Thus we have satisfied ourselves that small quantities of pregnandiol can be made to appear in the urine after progesterone injection under conditions in which endogenous pregnandiol is not to be anticipated. The recovery has been small in every case and does not seem to depend upon the

degree of œstrinization of the endometrium. It is possible that larger injections might give a larger percentage recovery in the urine. The fact that on all three occasions on which pregnandiol has been found in the urine after progesterone injection, it has not been recovered until the fourth day of injection, suggests that there may be a kind of saturation phenomenon comparable to that which is now well known to occur in the excretion of ascorbic acid taken by mouth. If this should prove to be so then a longer continued course of progesterone injections, even if the daily dose is not increased, should result in a much higher percentage yield. It is possible also that some of the injected progesterone was excreted in the form of free pregnandiol, for the uncombined form is not estimated by the method of Venning which we have used in this work. We have not attempted to estimate free pregnandiol in these experiments, nor so far as we are aware have any such estimations been reported after progesterone injection.

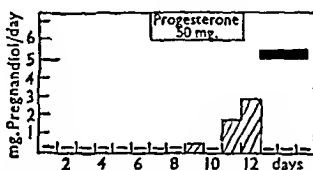


Fig. 13. Pregnanndiol excretion after injection of 50 mg. of progesterone in a case of anovular menstruation. Black rectangle marks uterine bleeding.

It is clear that there is much disagreement over the effects of progesterone injection on pregnandiol excretion, no workers having been able to recover yields as high as those reported by Venning and Browne. It is desirable that these differences in result should be reconciled by further attention to the conditions under which the experiments are made.

Amenorrhœa.

The proof that pregnandiol excretion is due to corpus luteum activity must include evidence that under all conditions in which the rhythm of the human luteum activity is suspended, pregnandiol ceases to make its periodic appearances in the urine. Its suspended activity may not always be recognizable but there are states in which it is regarded as reasonably certain.

Kurzrok (13) has produced evidence that secondary amenorrhœas may be the result either of hypofunction of the ovary without pituitary disorder, or alternatively of ovarian hypofunction secondary to pituitary hypofunction. In both types what we may call, by analogy with the famous neurological conception of Sherrington, the "final common hormonal path" is through the ovarian follicle and its corpus luteum. It would seem reasonable to suppose that in both these types the rhythmic ripening of ovarian follicles is in abeyance.

The first case of secondary amenorrhœa investigated was a young unmarried woman of 22 years, admitted to hospital because of a ten months' history of hyperthyroidism of mild degree. The waking pulse rate varied between 80 and 100 and the basal metabolic rate between $+14\%$ and $+22\%$. The menstrual history was normal. Menarche occurred at 14 years. A 24 day rhythm with 5 to 6 days bleeding was established and continued regularly up to the time of admission to hospital. Although pelvic examination was necessarily incomplete, there was no indication of genital abnormality. The breasts were normally developed. Three days after admission to hospital a normal menstrual flow started and continued for 5 days. Two days after this bleeding ended pregnandiol estimations were begun on the urine. Twenty-four hour samples were collected daily, and mixed two day specimens were analysed as soon as received. It was anticipated that menstrual bleeding would again recur after the customary 24 day interval and that pregnandiol would appear in the urine about one week before this, but no trace of pregnandiol appeared at the expected time, nor did any menstrual bleeding follow. Pregnanliol analyses were continued on all urine collections for a total period of 33 days and in none was any pregnandiol found. The period of amenorrhœa, thus quite unexpectedly begun, persisted for several months.

The second case was a young girl of 17 years, admitted to hospital because of a three months' history of rapid loss of weight and severe anorexia, diagnosed as anorexia nervosa. There had been an attack of acute rheumatic fever one year previously without lasting cardiac involvement. Menses had commenced at 13 years and had continued at regular intervals of 28 days with four days flow until four months before admission to hospital when complete amenorrhœa began. In hospital weight was rapidly gained; she was not thought to be suffering from Simmond's disease. Pooled two or three day collections of urine were analysed over a total period of 44 days in this patient and in none of the 21 specimens was pregnandiol found.

The absence of pregnandiol from the urine of these cases of amenorrhœa over long periods is thus in full accord with the view that pregnandiol excretion is a manifestation of corpus luteum activity, for it is generally conceded that in cases of this type ovulation is in abeyance. Our negative findings in these two cases provide an adequate contrast to the pregnandiol excretion of normal menstruation. We do not imply that pregnandiol is absent from the urine in all types of amenorrhœa. The study of a much wider variety of such cases is clearly needed before such a point can be securely established. For instance, existence of amenorrhœa apparently does not preclude the ovulation and conception and it is possible that in such cases pregnandiol will be found in the urine.

Non-ovulatory bleeding.

A disorder, in which serious disturbance in the corpus luteum rhythm appears to be established, is to be found in the group of menorrhagias in

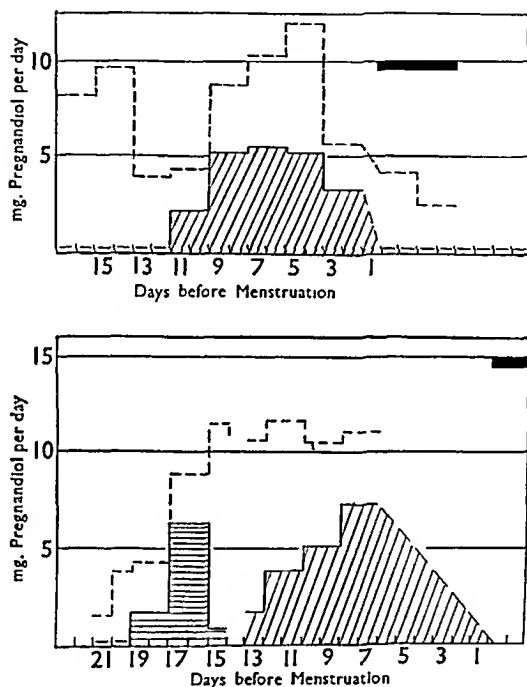
which the endometrium persistently fails to develop a secretory state. This group of uterine bleedings is regarded clinically as being of the non-ovulatory type and includes as an extreme form the clinical condition commonly called "*metropathia hæmorrhagica*." It is associated frequently with irregular and persistent uterine bleeding, sometimes so severe as to lead to serious anæmia. Although the careful investigations of Wilson and Kurzrok (27) and more recently of Israel and Mazer (11) suggest that the group is by no means a uniform one, it is generally believed that there is a lack of normal balance between follicular hormone and progesterone, in which the former continues to preponderate through what should be the luteal phase of the cycle.

The evidence on which this view is based is found both in the ovary and in the endometrium. The ovaries may show several small cysts, persistent unruptured Græfian follicles. Although the granulosa layer which lines these follicles may show signs of early luteinization, it is more often degenerate, and fully developed corpora lutea are never found. The appearance of the endometrium, though subject to considerable variation, shows also characteristic features. It tends to be thick and irregular and may contain blood clots. The glands, which are frequently well developed, are very irregular. Cystic dilatations may be present and when large impart to the section the characteristic so-called "swiss cheese" appearance. It is typical of these glands that although they may show considerable proliferation, yet they never develop the secretory changes which normally take place during the luteal phase of the menstrual cycle. A very similar endometrial picture to that seen in this condition can be produced experimentally by large doses of œstrin.

The condition may be regarded as one of an excessive and abnormally persistent proliferative phase of the usual endometrial cycle, whilst the absence of luteinization in the ovaries and of secretory changes in the endometrium indicates a gross inadequacy of the normal progesterone metabolism.

No studies of pregnandiol excretion in cases of this type appear yet to have been reported. We have studied one. The patient was a young unmarried woman of 21 years. Her history contained nothing of note until the age of 16 years when the periods, which had been considered normal for 2 years previously (menarche age 14), began increasing in frequency and abundance, though without dysmenorrhœa. With the onset of menstrual abnormality she began to gain weight; both continued until the time of admission. Whilst she was under observation bleeding occurred every 12 to 14 days and lasted from 2 to 6 days. Two days after the onset of uterine bleeding a curetting yielded much blood clot and surface epithelium, similar in appearance to the endometrium normally shed in menstruation except that the gland tubules showed no evidence of secretion. There was no cystic dilatation of these tubules.

During a period of 32 days all 24-hour specimens of urine were saved and two or three day mixed samples were analysed for pregnandiol. Three periods of uterine bleeding occurred during this time, but no pregnandiol was obtained from any sample of urine. Further analysis of all urine collections on the five days preceding another uterine bleeding one month later, again gave negative results. This case was the first we had seen in which "menstrual" bleeding was unassociated with recognizable pregnandiol in the urine.



Figs. 14 and 15. Pregnandiol excretion in a case of sterility and scanty menstruation showing evidence of independent excretion of other unidentified substances (*see text*). Interrupted line: weight of first acetone precipitate, expressed as pregnandiol. Obliquely shaded area: true pregnandiol excretion. Horizontally shaded area: atypical excretion product. Black rectangle marks menstrual bleeding.

The diagnosis of a competent gynæcologist on clinical grounds and from the endometrial biopsy had been "anovulatory menstruation." Our failure to find any pregnandiol in the urine of this patient thus provides evidence that a rhythmic uterine bleeding does not itself initiate the excretion of pregnandiol, and that when such bleeding occurs without the aid of corpus luteum activity, it is unassociated with the excretion of pregnandiol.

It is clear then that pregnandiol excretion is not a necessary accompaniment of uterine bleeding, and so is not associated with the hypothetical "bleeding factor" postulated by Kurzrok.

Atypical excretion products.

It was said earlier that in our view, certain forms of precipitate appearing in the first acetone precipitation by the Venning method do not represent true pregnandiol glucuronide, although they possibly contain a proportion of this compound. Venning (22) in her wide experience of the method also appears to have encountered such atypical precipitates. In one case of ours it has been possible to obtain evidence that such an atypical compound was excreted during the menstrual cycle, and that its excretion curve had a different time relation to this cycle from that shown by pregnandiol glucuronide, which nevertheless in this case followed a normal course.

The patient, a woman of 27 years, had been married for two years and was admitted because of sterility. Menstruation began at 13 years and continued at monthly intervals for two years. From the age of 15 until the end of the first year of married life periods had only occurred at two or three month intervals. During 1938 there had been periods only in January, May and December and each had lasted 5 days. Since May of that year she had increased 35 pounds in weight and her hair had become thinner. Another period occurred in January, 1939, but no further bleeding had taken place when she entered hospital in April, 1939. She was found to be a very phlegmatic, almost lethargic, individual. The excess of fat was mainly on the trunk. The breasts were well developed. The basal metabolic rate was within normal limits and the blood pressure 160/95. No other physical signs were found except a small uterus which had a two inch cavity and a rather scanty endometrium.

Routine pregnandiol estimations were begun on all 48 hour specimens from admission. For the first six days these showed a high level of substances appearing as the first acetone precipitate, but no pregnandiol glucuronide in the second acetone precipitation. In the specimen of the seventh and eighth days true pregnandiol glucuronide appeared for the first time, and this then followed a normal excretion curve, falling to zero eleven days later when a normal menstrual period began. An endometrial biopsy one day before this period showed an early secretory state, the glands being straight with slight convolution in a few zones. The glandular epithelium was hypoplastic, many areas were ciliated whilst in other zones secretory vacuoles were present at the bases of the cells. There was no vascular congestion but a few areas of interstitial hæmorrhage. The pathologists summary was "hypoplastic endometrium showing slight evidence of secretion." During this luteal phase 43 mg. of actual pregnandiol was recovered from the urine in the form of the glucuronide. These results are illustrated graphically in Fig. 14 in which true pregnandiol glucuronide excretion is indicated by the obliquely shaded area, whilst the weight of the first precipitate is shown by the interrupted line. Both curves in this and the following figure have been corrected by the usual factors to indicate excretion per day expressed as free pregnandiol.

Because of the unusually heavy first precipitate in the absence of true pregnandiol in the pre-luteal stage of this cycle, we were encouraged to follow the phenomenon during the succeeding month, during which Antuitrin S. was being given. Once more we were rewarded by a repetition of the same events. A well marked rise in the weight of the first precipitate occurred seven days before pregnandiol glucuronide appeared in the second acetone precipitate. Six days after its first appearance the true pregnandiol glucuronide excretion had risen to over 7 mg. per day, expressed as free pregnandiol, and this was followed in due course by a second menstrual bleeding (Fig. 15).

The several first precipitates obtained during the first six days of this series of estimations, on solution in 2 cc. of water had failed to produce any second precipitate in 98 per cent. acetone after standing overnight in the cold store according to the routine procedure, and so contained no typical pregnandiol glucuronide. These solutions in 98 per cent. acetone were then allowed to stand for ten days at $+3^{\circ}\text{C}$ when they gradually developed a fine fat-like precipitate which was quite unlike pregnandiol glucuronide and adhered firmly to the sides of the vessel. From the supernatant acetone layer no further yield of this substance could be obtained by evaporation.

The quantities of this substance so obtained, after having been arbitrarily corrected by the factor used for expressing pregnandiol glucuronide as its free pregnandiol equivalent, have been charted in Fig. 15 as the horizontally shaded area. It will be noted that the excretion of this substance, after a rapid rise, sank to a low level before pregnandiol glucuronide appeared. Although we have not been able to determine the nature of this fatty precipitate we have compared some of its properties with the actual pregnandiol glucuronide obtained from the same patient later in the same menstrual cycle. Its melting point was 211°C , compared with 257°C for the unrecrystallized pregnandiol glucuronide sample, and there was no evolution of gas. It contained approximately only 7 per cent. less glucuronic acid than did the pregnandiol compound and presumably was also a glucuronide. On hydrolysis with N/4 hydrochloric acid at 100°C for 30 minutes a brownish crystalline precipitate was obtained melting at 165°C , whereas the pregnandiol glucuronide sample treated in the same way yielded a pure white crystalline precipitate of pregnandiol melting at 213°C . Recrystallized pregnandiol glucuronide hydrolysed under the same conditions deposited free pregnandiol with melting point 227°C .

The conditions under which this substance appears in the urine are not, of course, to be deduced from one case. We have not observed appreciable amounts of it in the normal menstrual cycle, nor has it been present in cases of amenorrhœa. The details of this atypical case are noted here because they indicate that other substances may be excreted rhythmically during the menstrual cycle under conditions at present undefined. The observations further show clearly that in studying pregnandiol excretion no reliance can be placed on the weights of the first acetone precipitate, and fallacious

results may be obtained if the analysis is not carried through to the weighing of the precipitate after a second acetone precipitation.

Discussion.

The observations on pregnandiol excretion reported in this paper confirm the main claims of Venning and Browne that pregnandiol is excreted in the urine only during corpus luteum activity.

It has been shown that excision of the corpus luteum during early pregnancy results in a rapid fall to zero of the pregnandiol excretion, which normally continues in increasing amounts to full term.

We have confirmed in several patients that pregnandiol is excreted during the luteal phase of the menstrual cycle, that it falls to zero before bleeding commences, and that it is absent from the urine during the first or pre-ovulatory half of the cycle. In contrast to this it has been shown that no such excretion of pregnandiol occurred over a period of several weeks in two women suffering from secondary amenorrhœa, in whom it was considered unlikely that ovulation was occurring.

A woman has been studied who suffered from excessive uterine bleeding, and in whom a clinical diagnosis of non-ovulatory bleeding had been made. The fact that no trace of pregnandiol was excreted by this patient during a time which included three uterine bleedings provides additional evidence of the close association between corpus luteum activity and pregnandiol excretion.

But although this is all compatible with the main thesis of Venning and Browne concerning the significance of pregnandiol excretion, it must nevertheless be borne in mind that activity of a corpus luteum is usually associated in non-pregnant cases with a secretory type of endometrium. The available facts show with equal clearness the close association between pregnandiol excretion and a secretory endometrium, and so are equally compatible with the tenable view that such an endometrium is concerned in the origin of the pregnandiol. The well recognized fact, noted earlier, that the corpus luteum of pregnancy may be removed at the second or even the first month without abortion necessarily occurring, has led to the assumption that under these conditions the chorionic villi take over the hormone producing function of the corpus luteum. Since this seems very probable, then, we feel, the adoption of a similar endocrine function by the normal premenstrual secretory endometrium is by no means an impossibility. Further experimental work designed to distinguish between these two possible sources of origin of pregnandiol is clearly needed if its significance is to be more accurately defined.

Although we have been able to confirm that pregnandiol can be made to appear in the urine after injection of progesterone, yet our recovery of the substance has in all cases been small. That such a small yield is more frequent than the relatively high ones obtained by Venning and Browne is probable in that neither Hamblen, Ashley and Baptist nor Stover and Pratt were

able to recover a recognizable quantity. Moreover, in one of our cases 30 mg. progesterone was injected during five days without any pregnandiol being recovered from the urine.

In view of this great variability in the yield of pregnandiol from a given injection of progesterone, it is clearly unjustifiable to assume that the magnitude of pregnandiol excretion necessarily gives any quantitative indication of the rate of progesterone production by the corpus luteum. Though it is possible that endogenous progesterone is excreted more completely as pregnandiol than is injected progesterone, there is at present no reason for assuming this to be so.

It is possible that the state of the endometrium will prove an important factor determining the percentage yield of pregnandiol from a given quantity of progesterone. Our observations have not supported the view that oestrinization of the endometrium produces a larger yield, but it is realized that in this one case the endometrium could not be regarded as normal in other respects.

But the possibility should be kept in mind that pregnandiol excretion curves may reflect the state of the endometrium more closely than the activity of the corpus luteum.

Until more definite evidence to the contrary is produced, it must be assumed that the observed pregnandiol excretion represents only a small proportion of the total endogenous progesterone production. It is therefore desirable to enquire what evidence we have at present that pregnandiol is the most important excretory product of endogenous progesterone.

Numerous attempts have already been made to detect progesterone like activity in the urine with but little success. The work of Loewe and Voss (14) suggests that in neither the luteal phase of the menstrual cycle nor in the second month of pregnancy does the quantity exceed one rabbit unit per 20 litres of urine. From this and other similar observations it may be concluded that even under conditions of considerable corpus luteum activity no appreciable amount of progesterone appears in the urine.

From comparison of the formulæ of progesterone and pregnandiol it will be seen that transition of the former into the latter entails the conversion of two ketonic groups into hydroxyl. Theoretically therefore there should be an intermediate stage in which one group has become hydroxyl whilst the other remains ketonic. At least two compounds of this type are known, corresponding to the intermediate stages in the production of pregnandiol and allo-pregnandiol. These are respectively epi-pregnanol 3-one 20 and epi-allo-pregnanol-3-one-20. Marker, Kamm and McGrew (15) have sought these two compounds in pregnancy urine, and were able to obtain only doubtful traces of the former from 40,000 gallons of urine, whilst the latter was present in a concentration of 1 to 2 mg. per gallon. It is clear therefore that these intermediate compounds are present in an amount not exceeding two per cent. of the pregnandiol excretion, and so for practical purposes can be ignored.

The method of Venning for estimating pregnandiol glucuronide does not take into account free pregnandiol which, being easily soluble in acetone, is lost during the precipitation of the glucuronide. For the glucuronide estimations to have quantitative significance in relation to progesterone metabolism it is necessary to be certain that no large part of the pregnandiol is present in the uncombined state. The precise proportions of the free and combined forms do not yet appear to have been established, although the very much higher yields of the glucuronide extraction indicate strongly that the greater part is in this form. But Beall (2) studying concentrates of pregnancy urine obtained by benzoic acid absorption found 40 to 50 per cent. of the pregnandiol present in the free form, and Odell and Marrian (17) obtained 46 mg. of free pregnandiol from 100 litres of a urine from which they also obtained 110 mg. of combined pregnandiol. It is unlikely that these represent the proportions existing in the original urines, however, since both were pooled samples and it is known that hydrolysis rapidly sets in at room temperature unless special precautions are taken to prevent it. Until more accurate methods of estimating free pregnandiol become available the actual percentage present must remain uncertain, though it is probably small in most urines. A further possibility not yet fully investigated is the presence of the isomeric form allo-pregnandiol in appreciable quantities. There seems to be no doubt that this substance is present in pregnancy urine. Beall (2) has been able to isolate it in relatively pure form from benzoic acid concentrates of pregnancy urine after hydrolysis, although the yield was considerably lower than that of pregnandiol, and Marker, Kamm and McGrew (15) as mentioned previously, have obtained evidence of the existence in such urines of traces of allo-pregnane-3-ol-20-one, the intermediate compound between allo-pregnandiol and progesterone.

The available evidence, therefore, though admittedly still incomplete, does not reveal in urine appreciable quantities of any substance other than pregnandiol glucuronide and allo-pregnandiol, probably also present as a glucuronide, which might be considered a break down product of progesterone metabolism.

For the reasons given above, we feel that the evidence presented in this paper, taken in conjunction with the original results of Venning and Browne, and with those of other workers, justifies the conclusion that pregnandiol excretion is a qualitative indication of corpus luteum activity in the non-pregnant woman and that it is probably an indication of progesterone production in both pregnant and non-pregnant. We do not consider that there is any justification for assuming that the pregnandiol excretion rate provides any reliable estimate of the intensity of corpus luteum metabolism or of the rate of production of endogenous progesterone.

The great discrepancy between the relatively large recoveries of pregnandiol reported by Venning and Browne (24) after progesterone injection and the failure to recover any pregnandiol after similar injections by Hamblen, Ashley and Baptist (10) and by Stover and Pratt (19), together

with our own intermediate recoveries reported above, suggests strongly that other factors, at present undefined, are concerned in determining the pregnandiol excretion from a given quantity of progesterone.

If this be regarded as probable in the case of injected progesterone, it must be considered equally probable for endogenous progesterone.

Because of this doubt, great caution must be exercised in making deductions from variations in pregnandiol excretion under different circumstances. It is unjustifiable to interpret such variations as reflecting necessarily similar variations in progesterone production or in corpus luteum activity. In this respect we believe that the influences of renal and hepatic efficiency will prove to be of minor importance. Renal impairment must become very severe before the daily output of excretory products becomes seriously affected, although their concentration may rise in the blood. Similarly it is generally believed that an advanced degree of liver damage must be present before hepatic function is seriously affected. It is significant that among the cases in which pregnandiol excretion after progesterone injection has been studied or sought, there has been no report of renal or hepatic inadequacy to account for the widely varying yields.

We believe that other hormonal influences, and the metabolic state of the uterus and endometrium will prove to be important in progesterone metabolism and pregnandiol excretion.

We feel that although pregnandiol excretion may be regarded as a qualitative indication of corpus luteum activity, yet the assumption that it bears any quantitative relation to such activity is at present unsupported by evidence, and is indeed contrary to the scanty observations so far made.

SUMMARY.

(1). A series of observations is reported which fully support the general thesis that pregnandiol is an excretion product of progesterone metabolism, and so is associated with activity of the corpus luteum.

(2). Pregnanndiol is excreted during the luteal phase only of the normal menstrual cycle, and this excretion ceases before bleeding occurs.

(3). Excision of the corpus luteum in early pregnancy leads to a prompt cessation of pregnandiol excretion.

(4). Injection of progesterone leads to a small but definite excretion of pregnandiol in women not previously excreting this substance.

(5). No pregnandiol is excreted in secondary amenorrhœa.

(6). No pregnandiol was excreted during 32 days by a woman with non-ovulatory bleeding in whom it was believed corpus luteum activity was in abeyance.

(7). Evidence has been obtained of the excretion of another glucuronide rhythmically during the menstrual cycle in one case.

(8). It is considered that the assumption that pregnandiol excretion reflects quantitative variations in corpus luteum activity is unsupported by direct evidence and seems unjustified.

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VENOUS PULSATION IN THE ORBIT.

By T. LEWIS.

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WHEN, in cardiac failure, the venous system is engorged, pulsation of the veins is often very prominent and extensive, as is well known. In such cases careful observation will often reveal its extension as far as the elbow or even into the veins of the forearm, and very occasionally it may pass back as far as the dorsum of the hand. Similarly it extends through the veins of the neck, and I recall one case seen many years ago in which prominent pulsation was present in large superficial temporal veins and in veins on the top of the scalp.

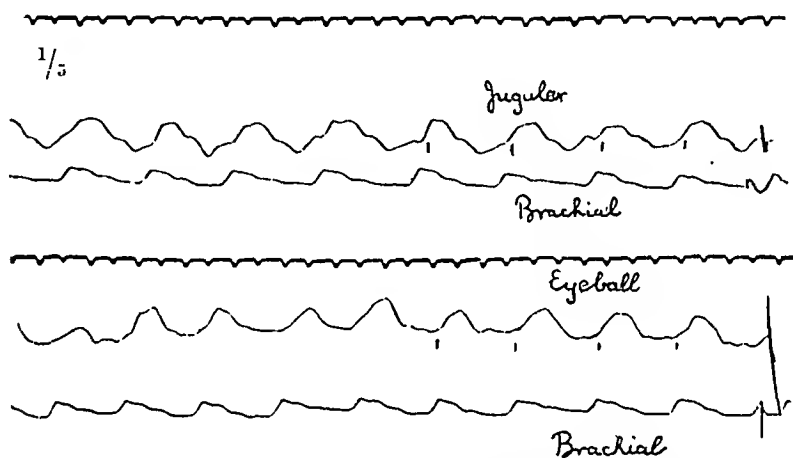
Such pulsation is maximal at the level at which the veins tend to collapse. Thus the venous pulse at elbow or in hand is elicited by bringing the corresponding part to an appropriate level relative to the heart, namely, a little above it.

A few years ago a woman of 56 was admitted under my care suffering from aortic disease and advanced cardiac failure from which she died. The heart was a large one (700 g.) the aortic valves were thickened, retracted and incompetent, giving distinct water-hammer pulse during life (S.B.P. 156). The ascending aorta and arch though not much dilated presented advanced syphilitic changes. While under treatment she was dropsical, the liver was enlarged, and the veins of the neck prominent and pulsating to the angle of the jaw with the woman reclining on many pillows.

The excess pressure in the veins was estimated at about 11 cm. of water, and it seemed to vary little under treatment. The pulse was slightly irregular, auricular fibrillation being present and the ventricular rate controlled by digitalis.

While the woman reclined in bed at about 45°, the veins of the neck to the jaw became so engorged that they ceased to pulsate; the pulsation now occupied a region extending from the angle of the jaw to the front of the ear and the whole region of the temple pulsated, as did a superficial vein coursing across it. With each pulsation of this region a very definite forward movement of the eyeball was visible, a movement of several mm. in extent; but like the pulsation of the temple, the upstroke was too slow and the movement too soft to be felt.

A polygraphic record of the jugular and brachial pulses is shown in Fig. 1. The pulse is slightly irregular and its rate 84 per minute; and the venous pulse is ventricular in form. A small funnel of suitable size was fitted over the right eyeball and the corresponding curve is shown with a brachial pulse record in Fig. 2. It does not differ substantially from the jugular curve, except that its upstroke is perceptibly delayed, when compared with the latter. The amplitude of this orbital pulsation was maximal in the posture described, it decreased when the patient was placed in a more upright or in a more reclining position, as is frequently, indeed



Figs. 1 and 2 ($\times \frac{1}{2}$). Polygraphic records from brachial artery, jugular pulse, and eyeball.

usually, the case where venous pulsation is concerned. With the patient in the same posture the veins of the fundus oculi pulsated very vigorously in systole, the veins being greatly engorged in systole and almost completely collapsed between the beats.

Since this observation was made 5 years ago I have looked for the sign in other cases of congestion, but have seen none in which it was nearly so prominent. Pulsation that is just visible is not very infrequent, if the observed eye is brought to a suitable level relative to the heart; but it must be looked for closely to be observed. It arises presumably in the veins at the back of the orbit.

OBSERVATIONS ON PERIARTERITIS NODOSA.

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As may be seen from the reviews that have appeared from time to time our knowledge of periarteritis, or better polyarteritis, nodosa is scant (17, 27, 32, 41, 44, 56). It is generally regarded as a rare disease, with the character of an infection or toxæmia, that almost always ends fatally after a course lasting on an average from four to five months and, because of the bewildering variety of the clinical manifestations, is seldom recognised during life. Thus of the previously published cases, about 350 in all, the diagnosis was made during life in only about 50, and in many of these it was made more or less accidentally after microscopical examination of excised tissue. In 1937 Leishman (41) published a clinical account of four cases recognised at autopsy in St. Bartholomew's Hospital, together with an analytical survey which he hoped might serve as a foundation for the clinical diagnosis of the condition. It is of interest, therefore, to record my experience at this hospital which strongly suggests that the condition is much less rare than is commonly thought, that it is not necessarily or even usually fatal, and that it can be recognised at the bedside in a considerable proportion of cases. Since the end of 1937 seven cases have been recognised; of these only three have died. The diagnosis was made on clinical grounds, and confirmed histologically, in four, (Cases 2, 3, 4 and 5); in two others (Cases 1 and 6) it was suspected during life; in the remaining Case 7, it was discovered after death. In addition several other cases are, or have been, under observation in which the clinical picture strongly suggests the diagnosis but for which histological confirmation has not been obtained.

Leishman (41) suggests that the rarity of the diagnosis must at least in part be due to unfamiliarity with the disease. My own experience bears this out. Acquaintance with the general features later outlined teaches that many of the cases present a complex combination or succession of symptoms that sooner or later suggests the diagnosis. Before recording my cases, I describe briefly the chief diagnostic features of the condition, both

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I wish to acknowledge help received from my colleagues at Guy's Hospital, specially from Dr. F. N. Glover and Dr. Alfred Vogl, in making observations on patients and in reviewing previously recorded cases.

histological and clinical, on the basis of my own experience and of a review of the majority of the previously published cases. A similar condition occurs in animals, such as the pig, calf, dog and deer (6).

Pathology.

The pathology of the condition was well described by Gruber in 1926 (27) and but little has been added since. The chief feature is what is variously called a necrotising arteritis or a hyaline or fibrinoid necrosis* of the arterial wall together with a surrounding inflammatory reaction. The necrotic portion of the vessel wall stains a bright pink with eosin and is conspicuous in sections stained with this and hæmatoxylin. The necrosis affects mainly the inner part of the media and the subintimal regions (Figs. 8 and 11) but the whole thickness of the wall may be involved (Figs. 1 and 14); it affects usually only a segment (Figs. 2, 3 and 8) but sometimes the entire circumference† (Figs. 1 and 14). Only short stretches of the vessel are attacked (Fig. 11) and specially at points of branching. The fibrinoid material may enter the adventitia (Figs. 2 and 14) but more usually it accumulates beneath the endothelium and, lifting this before it, encroaches on or obliterates the lumen (Figs. 8, 11 and 12). The affected portion of the artery becomes disorganised and the internal elastic lamina breaks up (Figs. 2 and 3). As a result, aneurysms may form (Fig. 13) and hæmorrhage may occur into the wall of the vessel and the surrounding tissues (Fig. 7). Thrombi often form within the vessel. Frequently the adventitia and the surrounding tissues are swollen and cedematous (Figs. 2, 12 and 14), though they may show little change. The type and degree of the inflammatory cellular reaction varies greatly and apparently depends partly on the type of case and the stage of the disease at which the tissues are examined. At one extreme so densely is the affected part infiltrated with polymorphonuclear leucocytes that the picture is almost that of a purulent inflammation (Figs. 1, 2 and 14); at the other there is but little sign of tissue reaction. Usually, however, the cellular inflammatory reaction is conspicuous and is subacute in type with not only polymorphonuclear leucocytes, but also mononuclear cells, lymphocytes and plasma cells. Giant cells of the foreign body type are sometimes present (Fig. 8). Eosinophil cells, sometimes in large numbers, are also a characteristic though inconstant finding (Case 4). In most cases some of the

* This fibrinoid necrosis is to be distinguished from the hyaline degeneration frequently found in older subjects and specially in cases of benign hypertension. Even in advanced hyaline degeneration the outline of the tissue elements affected and their nuclei are often remarkably well preserved; in the necrotic lesion the tissue elements are early disorganised, their outlines disappear and the nuclei break up; the necrotic material is rather cloudy or faintly granular than hyaline in appearance and stains more intensely with eosin. The distinction as a rule is easy but in cases where both lesions are present (as in Case 7 of this series) transitions between the two types of lesion are to be found suggesting that the necrosis represents but a more active grade of the degenerative process, (see also Klempner and Otani (36)).

† To display the relation of the necrotic zone to the elastic tissue I have found it useful to stain sections first with Weigert's elastic stain and then, after thorough decolourisation, to stain with Ehrlich's acid hæmatoxylin and eosin. Weigert's stain is most selective when freshly made.

lesions show signs of healing in the form of granulation or scar tissue (Figs. 3 and 10). As a result the vessel wall becomes thickened, locally or generally, with splitting of the internal elastic lamina (Fig. 5), fibrosis of the media and adventitia and intimal hyperplasia; one or more new vessels may be formed within the original lumen (Fig. 10). It is to be noted, however, that there seems to be no feature of these healing lesions to distinguish them from the healing stages of other forms of arteritis; in the present stage of our knowledge the presence of both fibrinoid material and of a frank inflammatory cellular reaction is required for the diagnosis of periarteritis nodosa. The finding of acute subacute, healing and healed stages in a single case agrees with the clinical evidence that the lesions tend to occur in crops throughout the course of the disease, which may last not only for weeks or months but for years. The necrosis affects the walls of the smaller arteries (for example the branches of the coronary and mesenteric arteries) and arterioles; the larger arteries are seldom involved and then mainly by way of their nutrient vessels.

The lesions may be visible to the naked eye as whitish nodular thickenings along the course of the arteries but the nature of the condition is often only recognised under the microscope. As a rule the lesions are found widespread though there seems to be a predilection for the kidneys and heart. They are often present in the alimentary tract (being specially well seen in the mesentery), and in pancreas, liver, spleen, skeletal muscle, peripheral nerves and skin; lesions have been found less frequently in the lungs and central nervous system. They may be numerous or sparse in any one part and in some cases seem to have been restricted to one or a few organs. It is to be remembered, however, that many cases have been recognised only after histological examination, and it seems likely that had the diagnosis been in mind earlier and a more careful search instituted, wider distribution would have been revealed. My own experience emphasises the necessity for examining excised tissues in serial section. For example, the first sections cut from the excised pieces of skin and the lymphatic gland from Case 4 failed to show the characteristic arterial lesions. The clinical picture, however, so strongly pointed to the diagnosis of periarteritis nodosa that the remainders of the blocks were cut in serial sections. In the lymphatic gland the arterial lesions were restricted to small vessels in the hilum while in the two pieces of skin only a single arteriole in one of them was affected over a short stretch of its course.

In addition to the arteries, the small veins are sometimes similarly involved while focal lesions of the minute vessels also occur. Though the wall of the minute vessels is disorganised, the characteristic fibrinoid change is not seen and the lesions show no special features to distinguish them from those of inflammation in general. In addition to these vascular lesions, small foci of parenchymatous degeneration or necrosis occur which do not seem to be directly dependent on local vascular lesions; examples are the necrotic foci in the skin of Case 4 (Fig. 9), and those in the myocardium of

Case 2 (Fig. 6), which closely resemble the submiliary nodules associated with acute rheumatism. Vegetations on the heart valves and elsewhere on the endocardium are recorded in a few cases.

With such vascular lesions varying in severity, number and distribution, the variability of the gross anatomical changes is an expected result; for example the degenerations, infarcts, necroses and fibroses and hæmorrhages occurring in different organs, the gastro-intestinal inflammations and ulcers, the various degrees of nephritis and nephrosclerosis, fibrosis of the liver, the nerve degenerations and the multiform cutaneous lesions.

We are ignorant of the cause of periarteritis nodosa. Some regard it as due to an unknown specific infection, a view often strongly suggested by the clinical picture; in the axis deer it occurs apparently in epidemic form (41). It is suggestive from this point of view that Baló and Nachtnebel (7) record three human cases recognised at autopsy within the space of 9 months in 1919 and that though others were watched for, none were seen until 1925 and then nine cases occurred in two years and nine months. On the other hand, the failure to detect any infective agent, the variety of the prodromal diseases, the association with such phenomena as eosinophilia, urticaria and asthma, and the evidence from animal experiment have led the majority of workers to conclude that the vascular lesion is not due to a specific disease but is rather the expression of a characteristic reaction of the arterial system possibly allergic in nature, occurring in the course of various infections and toxæmias (27 and 48). In animals a necrotising arteritis has been produced in a variety of ways, for example by the injection of bacteria or their toxins or of the antigens in a previously sensitised animal (2, 15, 46); Clark and Caplan (13) record such arterial lesions following large doses of antipneumococcal serum in man. Again, in animals a necrotising arteritis is caused by producing arterial hypertension by Goldblatt's method (23, 56, 57). Dr. Clifford Wilson has been good enough to show me sections prepared from rats suffering from hypertension provoked by clamping a renal artery. The arterial lesions, in the kidney, heart, stomach, pancreas, etc., in all their stages closely resemble those of human cases of periarteritis nodosa, and in his series of sections I was able to match all the different histological pictures seen in my own sections.* Wilson and Byrom believe

* While the kidneys of these rats display arteriolar and glomerular lesions closely resembling those found in human cases of malignant hypertension, yet the arterial lesions in general are widespread and show a conspicuous inflammatory cellular reaction. The necrotising arteriolitis specially found in malignant hypertension is not as a rule accompanied by a frank inflammatory cellular reaction like that of periarteritis nodosa and is almost confined to the afferent arterioles of the kidney (21). There are, however, certain cases of malignant hypertension, apparently superimposed on either a previous benign or a previous nephritic hypertension, in which inflammatory necrotic lesions of the arteries and arterioles are found in the kidney and elsewhere in the body, that are indistinguishable from those of periarteritis nodosa (20, 31, and Case 7 of this series). In some of these cases the inflammatory lesions are but sparse; in a case recorded by Goldbloom and Lieberman (25) only a single focus was found in the perirenal fat. Klemperer and Otani (36) point out, however, that in their two hypertensive cases displaying inflammatory vascular lesions like those of periarteritis nodosa, there was also present a morbid process generally recognised as toxic in origin, subacute glomerular nephritis in one and hyperemesis gravidarum in the other.

that the factor determining the lesion is probably a sudden strain imposed on the vessel wall by the combination of severe vasoconstriction and the resultant rapid rise in blood pressure. Although it is possible therefore that hypertension may be one factor in producing periarteritis nodosa yet the fact that most human cases of periarteritis nodosa do not display hypertension shows that some other cause must be at work. On the other hand, that some cases of periarteritis nodosa do develop hypertension is not unexpected in view of the frequency with which the lesions occur in the kidneys and of the experimental evidence for the production of hypertension by restricting blood flow to the kidney.

Some authors believe that periarteritis nodosa is more or less closely related to rheumatic fever. Friedberg and Gross (22) point out that the symptoms of the two conditions have a number of points in common and they record four cases in which not only the clinical picture suggested rheumatic disease but, after death, old and recent valve and myocardial lesions generally regarded as characteristic of rheumatic heart disease were found associated with the arterial lesions of periarteritis nodosa. They conclude that the periarteritis nodosa in their cases is an expression of the rheumatic infection. It is possible, however, to regard the so called rheumatic lesions as an expression of the periarteritis nodosa. Thickening of heart valves and verrucose vegetations are not specific for rheumatic carditis and, if it is remembered that the endocardium is comparable in structure to the arterial wall, it is not unexpected to find this involved in severe and widespread periarteritis nodosa. Again, the submiliary nodule of rheumatic fever is no more than the inflammatory reaction around a necrotic focus in the myocardium. In my own series, the clinical manifestations of Case 1 suggested rheumatic fever complicated by nephritis, yet no endocarditis or myocardial submiliary nodules were found after death; on the other hand, in Case 2, though the clinical picture was unlike that of acute rheumatism, yet both endocarditis and submiliary nodules were discovered post mortem (Figs. 4 and 6), while in Case 4, also clinically unlike rheumatic fever, foci of fibrous tissue necrosis were present in the skin (Fig. 9); inflammatory foci were also found in the heart in Case 7.

It is clear then that we have no adequate knowledge of the cause of periarteritis nodosa in man; in the meantime it seems best to regard the arterial lesion as an expression of injury to the vessel wall brought about by various factors not yet recognised. It is to be remembered that our present knowledge of the condition in man is largely based on examples recognised as such only after death, sometimes only under the microscope; it follows that the clinical, and to a less extent the pathological pictures so far drawn are necessarily incomplete. It is important therefore that we should be able to recognise our cases at the bedside and at the earliest possible stage of their course in order that we may better define the clinical pictures and attempt etiological classification. Our present knowledge is readily comparable to what we knew of endocarditis about the middle of the last century.

Then our conception of the very variable clinical manifestations was indefinite and confused and many cases were recognised only after death. Attempts to classify the cases from both clinical and pathological aspects were unsatisfactory. Now, we have learned to distinguish at the bedside, at autopsy, and under the microscope rheumatic endocarditis and bacterial endocarditis, acute and subacute (26).

Clinical manifestations.

In contrast to the essential unity of the underlying arterial lesion, the clinical manifestations of periarteritis nodosa are exceedingly varied; Leishman (41) remarks that they are distressingly protean. In fact it is difficult to find any two cases with the same course among those hitherto reported, about 350 in all. Almost the only simple statements that can be made about the disease are that it may occur at any period of life from childhood to old age and that it affects males much more often than females, the proportion being about 4 to 1. While in some cases the patients have previously enjoyed good health yet a remarkable feature of many is the frequency of earlier illnesses of very varied nature. It may begin insidiously or acutely and often appears to arise, more or less directly, from some other infective or toxic state which itself is obscure in origin. The prodromal symptoms are not uncommonly those of gradually increasing general debility, an upper respiratory infection, an "influenza," a "rheumatic" attack, a nephritis or of some visceral disturbance. When the very varied expressions of periarteritis nodosa itself are borne in mind, the thought arises in considering such cases that these previous illnesses may well indicate earlier manifestations of this disease; the time of onset of periarteritis nodosa is thus often uncertain, and so also is its duration.

A complete list of the symptoms of the established disease would cover almost the whole field of medicine, as would also a list of the diagnoses made before the nature of the condition was recognised. A brief enumeration shows clearly the varied nature of the clinical manifestations both general and local. It is to be remembered, however, that we are uncertain whether or not to attribute all these symptoms to periarteritis nodosa itself or to some other malady giving rise to it. Even the general symptoms of fever and leucocytosis can be regarded as due either to an infection or toxæmia, which is also the cause of the arterial lesions, or to the absorption of breakdown products from tissues deprived of their normal blood supply by the arterial disease. It is to be borne in mind also that secondary infections may develop in devitalised tissues, further complicating the clinical picture.

A low irregular fever is usual but the temperature may be of the septic swinging type, while some cases are afebrile throughout. The pulse rate is usually accelerated out of proportion to the degree of fever though it may be normal. Some degree of general wasting and weakness is usual but these may be either extreme or absent. Of the blood changes a moderate secondary anæmia and leucocytosis are most frequent. The leucocytosis, however,

may be high (40,000 to 60,000) ; more rarely there is leucopenia. Leucocytosis is generally due to an increase in the neutrophil polymorphonuclear leucocytes but eosinophilia, sometimes reaching high figures (79% in Strong's case (53)), is present in over 30% of the cases in which a differential leucocyte has been recorded. Lymphocytosis, as in Case 5, is also reported by Motley (47) and Spiegel (52). Many varieties of *skin lesions* occur ; simple erythema, erythema multiforme and nodosum, urticaria, purpura, cutaneous and subcutaneous nodules and vesicular, pustular or necrotic lesions (16, 34). Involvement of the *respiratory system* is shown by bronchitis, asthma, broncho-pneumonia, pleurisy and pulmonary infarction (31). *Digestive symptoms* may be no more than slight, with vague abdominal discomfort or severe pain, and point to an acute surgical condition such as appendicitis, a perforated ulcer or an intra-abdominal hæmorrhage. Inflammation and ulceration of the mouth and fauces occur, as also constipation or diarrhœa, often with blood and mucus. The *spleen* and *lymphatic glands* may be enlarged. Signs of *cardiovascular involvement* are generally limited to the tachycardia already noted though congestive failure is not uncommon ; endocarditis, pericarditis and anginal attacks are all recorded. Affection of the peripheral vessels may be evident in Raynaud's phenomenon with or without gangrene of the extremities (11, 12, 14). Hypertension is not uncommon and may develop during the illness. *Renal manifestations*, recorded in about 80% of the cases, may be no more than an occasional albuminuria to which may be added some hyaline or granular casts and a few red cells. There may, however, be all the signs of a severe acute or chronic nephritis, gross hæmaturia suggesting a surgical condition or gross renal failure and uræmia. The most common *nervous manifestation* is a peripheral neuritis (for example of the ulnar, radial or anterior tibial nerves), often with muscular palsy first and sensory loss following later and affecting one or all four limbs, not as a rule simultaneously but in succession. Cerebral and meningeal symptoms seem to be uncommon (54). Involvement of the *locomotor system* is shown by aches and pains in the muscles in which localised tender areas or nodules may be found and by swelling and tenderness of the joints. Muscular weakness and wasting have already been referred to.

It follows from what we know of the pathology of the condition, and it happens in practice, that the chief diagnostic features are local symptoms due to the inflammation of small arteries. It is obvious, however, that many of the local symptoms enumerated above may be conceived as arising in other ways and an arterial origin for them would probably not be considered unless the possibility of periarteritis nodosa was in mind. In fact the disease often presents itself as what Leishman (41) describes as an unusual and apparently unrelated combination of signs. It is for this reason that the discovery of such signs as nodules along the course of superficial arteries, pulsating subcutaneous nodules, obliteration of the pulse in small arteries, Raynaud's phenomenon due to arterial obstruction, gangrene of the extremities and anginal pain, particularly in the young, is of

special diagnostic importance since they clearly indicate arterial disease. The occurrence of hæmorrhage from the digestive tract, lung or kidney or into the skin indicates vascular but not necessarily arterial damage. Other symptoms such as peripheral neuritis, congestive cardiac failure, painful areas in the muscles and swellings of the joints, though they can be attributed to vascular damage can also be explained in other ways. Such manifestations as asthma and diarrhœa would not at first suggest vascular disturbance. Nevertheless, a combination of symptoms each of which can be interpreted as due to local circulatory disturbance is a strong indication for diagnosis, specially if one symptom at least of the combination can be shown to arise from involvement of the small arteries. The more parts of the body affected and the more certainly the symptoms can be attributed to circulatory disturbances and specially to arterial damage, the more assured does the diagnosis become. Thus for example in Case 4 among other manifestations there were the successive palsies of the arm and legs, the cutaneous lesions resembling the Osler's nodes of bacterial endocarditis, the occasional red blood cells in the urine, the bloody diarrhœa and ulceration of the rectal mucosa associated with clear signs of obstruction to the small arteries of the hands.

It is to be borne in mind that apparently any part of the body may be involved, and usually several or many parts to a differing degree, simultaneously or in any order of succession. The symptoms due to the involvement of one part may predominate, and thus point to the diagnosis of some local inflammatory condition. Not infrequently abdominal symptoms have led to operative interference, for example for appendicitis (Case 2), cholecystitis or a perforated ulcer; in Powell and Pritchard's (50) case hæmaturia led to removal of a kidney. In such instances microscopic examination of excised tissue usually reveals the arterial disease. But though the clinical picture may resemble that of some other better known disease there are usually additional features not readily accounted for on this basis, for example an acute nephritis in a case resembling one of rheumatic carditis, or an unusually high eosinophilia with asthma. A characteristic feature of the disease and one clearly illustrated by my cases is a changing clinical picture; fresh symptoms develop and older ones subside often to return again. Changing symptoms are emphasised by Emerson, Schroeder and Maynard (19) as specially important in diagnosis; the underlying pathology, they say, is seldom suspected at first, but after the patient has been watched for several weeks the bizarre picture and rapidly changing complaints make it clear that the process is a general rather than a local one.

The general picture of the established disease is sometimes that of an acute infection or toxæmia, like for example a streptococcal infection or acute rheumatic fever, with fever, leucocytosis and rapid prostration combined with some or others of the local manifestations enumerated above. Cases 1 and 2 are of this type; see also cases recorded by Friedberg and Gross (22), Arkin (4) and Leishman (41).

Most commonly, however, the general picture is that of a subacute illness, often with remissions or intermissions and acute exacerbations, characterised by low and irregular fever, tachycardia out of proportion to the degree of fever, leucocytosis, anæmia, progressive weakness and loss of weight combined with one or more of the local manifestations already mentioned. This form of the disease is one most readily recognisable and conforms to that of the two cases originally described by Kussmaul and Maier (38). It is of interest in this connection to recall that the diagnosis in their second case was made on clinical grounds alone and was suggested by the patient himself who had recovered from his illness. He had noted the resemblance of his symptoms to those of another patient (their first case) who was later admitted to the same ward and died. The chief symptoms of both were fever, tachycardia, weakness, pains in the limbs and body, paralysis of the limbs, abdominal symptoms and nephritis; in the fatal case enlargement of the lymph glands and subcutaneous nodules were additional features. Cases 3 and 4 of my series fall into this group and both were recognised on clinical grounds; similar cases are recorded by Bahrman (5), Curtis and Coffey (17), Lamb (39), Lewis (42), Macaigne and Nicaud (45), Motley (47), Otani (49), Strong (53), Weir (55).

In a small number of cases, general symptoms are but little in evidence, local symptoms being the chief manifestations of the underlying arterial disease. Thus in Case 5, fever, tachycardia and general wasting were absent and the patient was never seriously ill; the chief manifestations were confined to the left arm. Case 6 presented itself as a transient erythema multiforme of the hands and face, subcutaneous nodules appearing later; it recalls that recorded by Dietrich (18) in which an erysipelatous condition of the forearm was followed by the development there of subcutaneous nodules.

Lastly, in a small proportion of cases the main clinical picture is that of a severe or malignant hypertension and of these Case 7 is an example that was unrecognised during life. Similar cases are recorded by Bau (8) and Bernstein (11). In this group diagnosis during life presents special difficulties; an exploratory laparotomy led to it in the case recorded by Emerson, Schroeder and Maynard (19). According to Klemperer and Otani (36) the histories of such cases do not differ from those in which the periarteritis nodosa lesions are not found at autopsy. My own experience of a number of cases of the malignant phase of hypertension specially observed during life from this point of view shows that such general phenomena as loss of weight, fever, leucocytosis and anæmia and local manifestations such as nervous and abdominal symptoms, visceral and cutaneous hæmorrhages occur although careful search of tissues excised during life and after death fails to reveal the lesions of periarteritis nodosa. Nevertheless, in dealing with cases of malignant hypertension the possibility of periarteritis nodosa should be borne in mind with the view of discovering other local manifestations of diagnostic importance.

It has already been mentioned that periarteritis nodosa is generally regarded as a fatal disease running a relatively short course. In some cases, it proceeds steadily to a fatal termination within a few weeks, for example Cases 1 and 2, in which the total duration was no more than six weeks and two months respectively. In the majority of instances so far recorded, however, the illness pursues a more protracted course, often with periods of improvement and exacerbation, but usually ending in death after an average of from four to five months. Cases of much longer duration, not only with remissions but intermissions sometimes of considerable length, are recorded. For example Arkin (4), Jager (33) and Runge and Melzer (51) each describe cases of 4 years' duration, Kourilsky, Garcin and Bertrand (37) one of 8 years, Macaigne and Nicaud (45) one of 10, Ledoux* (40) one of 12, Heinrich (30) one of 14, and Berardinelli* (10) one of 32 years' duration. In many of the other cases recorded as of apparently shorter duration the onset might be considerably antedated by taking into account previous similar illnesses reasonably regarded as expressions of periarteritis nodosa. I have calculated in this way the average duration of 70 cases, unselected except for completeness of the recorded histories, as being just over two years. Of my own series it seems justifiable to date the onset of the disease in Case 3 to the "rheumatic" attack five years before her admission to hospital and to see in that attack and in the more or less continued ill-health subsequently the symptoms of periarteritis nodosa; the disease may thus be regarded as having lasted for no less than 8 years. In Case 5, the diagnosis was established in October, 1939, but the previous history warrants antedating the onset to at least the end of 1936 when the signs of peripheral vascular disturbance began; it is not unreasonable to see symptoms of periarteritis nodosa in the abdominal trouble of 1933 or even in the first asthmatic attacks of 1930. In Case 6, periarteritis nodosa was found during the course of an erythema multiforme; it is uncertain whether this represents its beginning or merely a recurrence, the first attack having expressed itself previously as a duodenal ulcer. It is not unreasonable to suggest that the long continued history of kidney trouble in Case 7 might have been due to periarteritis nodosa; it is more in keeping with recent experimental evidence to regard the periarteritis nodosa as a terminal phenomenon of pre-existing kidney disease which by reduction of renal blood supply had led to severe hypertension.

Further, it seems that the gloomy prognosis attached to the disease has arisen because the great majority of cases so far recorded have been recognised only after death. If we consider only those cases diagnosed during life, about 50 in all, then a half recover, at least temporarily. Because of the recurrent nature of the disease, records of recovery in the absence of a long period of after history must be regarded with reserve; but several cases are known in which the patient has remained well for several years at least

* Clinical diagnosis only, no biopsy.

(4 years, Harris, Lynch and O'Hare (28)). Of my own series of seven cases four are alive, though it is yet too soon to say that they have completely recovered. It is to be remembered, as Gruber (27) pointed out, that though arterial lesions themselves may heal the patient may continue to suffer or die from their effects. It seems likely, however, that as a greater proportion of cases comes to be recognised during life so the estimate of prognosis will improve, just as it has in the case of coronary thrombosis which until a few years ago was known only in the post-mortem room.

SUMMARY.

1. A series of 7 cases of periarteritis nodosa is described.
2. The chief pathological and clinical features of the disease are discussed.
3. The evidence suggests that periarteritis nodosa is much less rare than is commonly thought, that it is not necessarily or even usually fatal, and that it can be recognised at the bedside in a considerable proportion of cases.

CASE RECORDS.

Case I.

C.N., a boy aged 12, was admitted to hospital on November the 29th, 1938, with signs of cardiac and renal failure. He died on the eighth day, the total duration of his illness being about six weeks.

Except for whooping cough at seven and measles at ten years, the patient had been well until five weeks before admission. He then had a sore throat; a week later his knuckles and knees became reddened, tender and a little swollen and his legs ached. He was fevered (temperature 100 to 101°F.), short of breath, and suffered from a dry cough and frequent nose bleedings. His abdomen became swollen and tender; the frequent motions, about six a day, were loose and yellow. Since early in November his urine had been dark and muddy.

While in hospital his temperature remained between 100 and 102°F., falling terminally, the pulse rate being 130 to 140 and respirations 30 to 40 per minute. The veins of the neck were engorged; there was swelling beneath the eyes, gross ascites, slight oedema of the ankles, congestion of the lungs with bilateral pleural effusions. The heart was enlarged and a systolic murmur was heard at the apex. The blood pressure was 100 to 120 mm. Hg. systolic and 60 to 90 diastolic. Diarrhoea with loose unformed motions continued. The daily output of urine was about 16 ounces, the specific gravity being 1010. The dark and turbid urine contained much blood and many cellular and granular casts and pus cells; blood urea, at first 207, rose later to 280 mg. per cent.. No gross abnormality was detected in the nervous system but the patient was too ill for a thorough examination. His ocular fundi showed early swelling of the discs. No blood count was made. The patient steadily became worse and died suddenly after a vomiting attack on December the 6th, 1938.

The diagnosis was clinically uncertain but it was thought to be probably a case of rheumatic fever complicated by nephritis; this combination suggested the possibility of periarteritis nodosa, but the patient died before further observations were made.

At autopsy all the tissues were found to be grossly oedematous, and the serous sacs contained much straw coloured fluid. The heart was dilated, the muscle pale and oedematous, but no gross abnormality was noted in the valves or coronary vessels. The lungs were oedematous and hyperæmic but showed no signs of infection or infarction. The spleen was very large, weighing 410 g. and firm with prominent lymph follicles; the lymphatic glands generally were enlarged. The liver was not enlarged; it showed a fine regular granularity due to multilobular cirrhosis. Both kidneys were greatly enlarged (the left weighing 280 and the right 260 g.) and oedematous. Their surface was mottled; the capsule stripped readily. The pyramids were deeply coloured, the cortices congested and their normal pattern obscured. No abnormality beyond oedema was found in the other organs, including the brain.

The nature of the disease was revealed by microscopical examination of sections from the heart, pectoral musculo, liver, kidneys, and pancreas. In these, many of the arteries and arterioles showed the lesions characteristic of acute periarteritis nodosa. In the majority all the layers of the wall were more or less completely replaced by fibrinoid material and in many this had spread to the adventitia. In the majority also the inflammatory reaction was acute, the necrotic zone being invaded and surrounded by polymorphonuclear leucocytes (Figs. 1 and 2). Eosinophil cells, though present, were not a conspicuous feature. In some of the lesions the reaction had passed to the subacute stage as shown by the predominance of mononuclear cells and commencing proliferation of fibroblasts. In only a few had repair advanced to the formation of granulation tissue (Fig. 3). No giant cells were seen and no bacteria were found in any of the lesions. A number of small aneurysms had formed and in places also there were small hæmorrhages into the surrounding tissues. Similar inflammatory lesions were found in a few small veins.

In the kidneys the interlobular arteries and afferent arterioles were particularly affected. Some glomeruli showed necrosis of the capillary tufts with intracapillary hæmorrhage; a few also showed evidence of repair in proliferation of the capsule and tuft endothelium. The general picture was that of periarteritis nodosa and not of a primary glomerulo-nephritis.

The liver showed also a multilobular cirrhosis of recent origin, most probably the result of the arterial disease. In the heart, the myocardium appeared normal, no submiliary nodules being found. Sections from two cervical lymph glands, the lungs, spleen, the basal ganglia and mid-brain revealed no arterial changes.

Case 2.

A.R., a clerk, aged 20 years, was admitted to hospital on October the 16th, 1939, on account of an inflammatory condition of the gums, fever and vomiting. He died ten days later, the total duration of his illness being between two and three months.

Until recently the patient had enjoyed good general health. In August, 1939, while at a holiday camp, he noticed shortness of breath on exertion, on account of which he had to give up swimming; he began to lose weight. In the beginning of September he had a slight cold and noticed small raised areas on both upper and lower gums near the first molar tooth on the right side. On September the 7th, the lower right first molar tooth was extracted. The gums became more generally inflamed and bled when he cleaned his teeth. He attended the Dental Department of this hospital on September the 21st. His temperature was then 98.8°F.. The gums on the right side were inflamed as far as the mid-line. A swollen purplish area with a granular surface extended from the right lower canine to the right second molar; a similar smaller area was soon at the upper right first molar. A week later the condition had spread to the left gums; the jaw was not painful. His temperature was 97.8°F. and the blood pressure 130/85 mm. Hg.. No other sign of disease was noted; the lymphatic glands were not enlarged, the spleen was not palpable; there was no purpura; the reflexes, fundi, pupils, and urine were normal. A blood smear showed no abnormality; the number of leucocytes was within normal limits, polymorphonuclears forming 63%, lymphocytes 33%, hyalines 3% and eosinophils 1%; erythrocytes and platelets normal; no thrombocytopenia. A portion of inflamed gum was excised and examined microscopically; it showed septic granulation tissue covered with epitholium (for further details see later). By October the 13th, the gums had not improved; the patient complained of aching pains in his limbs, of nasal obstruction and bloody discharge, and of vomiting. His temperature was 100.2°F.. He was admitted to hospital two days later for further investigation. At that time the temperature had risen to 101.5°F.; the pulse rate was 125 per min., and the blood pressure 120/75 mm. Hg.. The gums were much inflamed, the mouth dry and dirty and the breath foul. The tonsils were enlarged. The nose was swollen and tender to the touch; its mucous membrane was hyperæmic and bled easily; the nasal cavities were blocked by firm blood clot. Another portion of inflamed gum was removed for microscopical examination.

The aching pain in the limbs continued; the pains affected the muscles of the shoulder, thighs and back; the muscles were not tender to palpation. The joints were neither swollen nor painful either to palpation or on passive movement. The bones were not tender to percussion. Respirations were rapid (35 per min.) and laboured. No abnormal signs were noted in the chest, abdomen, nervous system or blood. The urine (sp. gr. 1030) contained albumen and much blood. Venous congestion of 80 mm. Hg. applied to an arm for three minutes produced no petechial hæmorrhages. The patient vomited frequently. A number of firm small nodules were noted beneath the skin of the arms; the patient said these had always been there. On October the 17th, the general inflammation of the gums appeared to be subsiding. A swab from a small ulcer of the gum showed on direct smear desquamated epithelial cells spirochætes and few fusiform bacilli, but not in sufficient number to warrant a diagnosis of a primary Vincent's angina. A culture from the swab yielded a growth of streptococcus viridans with a few colonies of staphylococcus albus. A swab from the throat gave a practically pure growth of staphylococcus

aureus. The urino now contained only a trace of albumen, some granular casts and renal cells and very few red cells. Blood examination revealed hæmoglobin 72% and 9,800 leucocytes. The next day, the 18th, the patient's condition showed no improvement; vomiting, fever (101 to 102°F.) and tachycardia (pulse rate 120 to 130) continued. He was much distressed by the aching pains in body and limbs. In a search for signs of peripheral vascular disturbance a bruise was noted beneath the right great toe nail. A blood culture proved sterile. The blood Wassermann and Kahn reactions were negative. Treatment by M. and B. 693 was begun. On the 19th a blood count showed 12,000 white cells per c. mm., polymorphonuclears 87%, lymphocytes 7% and monocytes 8%; no abnormal cells were seen though many of the polymorphs were young cells. The urine contained albumen. On October the 20th, the patient was cyanosed and more breathless; the veins of the neck were engorged, and the heart and liver enlarged. A pleural rub was heard at the base of the left lung. Knee and ankle jerks were no longer obtainable; motor power was not affected. The generalised pain continued and again no areas of localised tenderness were found. The patient noted tingling sensations in his fingers on closing his hands. There was mucous discharge from the nose and throat. On October the 22nd, the patient coughed up bright red blood mixed with mucus. Two hæmorrhagic vesicles appeared on the dorsum of the 5th finger of the right hand, one 2 mm. and the other 6 mm. in diameter; they were only slightly tender to firm pressure. No other skin lesions were present. A second blood culture also proved sterile. Hæmoglobin had now fallen to 63% and the leucocytes increased to 20,000, polymorphs forming 92% (these showed marked granulation), lymphocytes 4%, and hyalines and metamyelocytes 2%. The urinary output was now greatly reduced having fallen from 51 ounces on the previous day to 16 ounces. On the following day the patient was gravely ill and weak; he was flushed, slightly cyanosed, and his veins still congested. His breathing was rapid (30 to 40 per min.). Both lung bases were dull and a widespread pleural rub was heard at the left base and râles and rhonchi throughout the chest. Ankle and knee jerks were still unobtainable; there was loss of appreciation of pain and sense of position in the legs. Vomiting continued. The urinary output was but 12 ounces and the alkaline urine contained albumen and red cells but no pus or casts. The blood urea measured 208 mg. per cent.. More hæmorrhagic vesicles developed on the hands and on the legs. One from the left patella, less than 24 hours old, was excised for microscopic examination. On both legs red tender streaks appeared along the course of the superficial veins. On October the 24th, further hæmorrhagic lesions appeared around the mouth.

A review of the patient's condition at this time strongly indicated a diagnosis of polyarteritis nodosa. The case presented the features of a severe infection or toxæmia of unknown origin, together with clear signs of cardiac and renal failure, with involvement of the lungs, the peripheral nerves, the musculature and skin. On October the 25th he became very restless, collapsed and died.

At autopsy the pleuræ and abdomen contained some serous effusion. The pericardium showed a fibrinous pericarditis and was adherent to the heart and the lower lobe of the right lung. The dilated heart weighed 310 g. The myocardium showed many small infarcts, and small greyish nodules along the course of the smaller coronary arteries. The endocardium seemed normal except for a group of small vegetations at the mouth of the inferior vena cava. The heart valves and aorta seemed healthy. The mucous membrane of the trachea and bronchi was congested; the trachea contained blood stained fluid. Both lungs were congested, mottled and oedematous and the left lower lobe contained a small infarct. Several of the small vessels on the cut surfaces stood out prominently and their walls were thickened. The stomach contained altered blood; the mucous membrane was not ulcerated. No abnormality was detected in the intestine or mesentery. The liver was enlarged, weighing 2,700 g., and showed fibrinous perihepatitis and early fatty change. The spleen was also enlarged, weighing 700 g., and covered with patches of fibrinous exudate. On section it contained much blood and was very soft and friable. Both kidneys were swollen and oedematous, the right weighing 310 and the left 300 g.; their capsules stripped easily and their surfaces were covered with small red flecks and showed scattered small greyish patches. On section many of the renal vessels showed small greyish thickenings. The medullary rays were prominent. No abnormality was detected in the adrenals, pancreas or thyroid gland. The brain and spinal cord were not examined.

Microscopical examination revealed arterial lesions characteristic of acute periarteritis nodosa in sections cut from the kidneys, heart, liver, spleen, mesentery and lungs. No lesions were found in sections from the left deltoid muscle, pancreas, thyroid and a cervical lymph gland. The arterial lesions closely resembled those in Case 1; there was, however, evidence of a longer continued process. More of the lesions showed a subacute reaction and there was a greater formation of granulation tissue. Eosinophil cells were more numerous and giant cells of the foreign body type were present. No bacteria were found in any of the lesions. Similar changes were present in the walls of the small veins some of which also contained thrombi, both recent, and in early stages of organisation. The arterial and glomerular lesions in the kidney were also the same as in Case 1, though many more glomeruli were involved. There were also areas of subcapsular ischemic necrosis. The picture was again that of periarteritis nodosa and not that

of a primary glomerulo-nephritis. The liver was congested and showed early fatty change but no cirrhosis. The splenic pulp was infarcted and necrotic. The lungs were congested and many alveoli contain desquamated cells; the arterial lesions here were sparse; most were found at the lung roots. The myocardium contains areas of infarction of different ages; in the majority the necrosis was in the early stages; a few showed beginning replacement fibrosis. Scattered throughout the myocardium were a few foci of granular debris surrounded by inflammatory cells (Fig. 6) which closely resemble the submiliary nodules associated with acute rheumatism. Several of these foci were present in the endocardium (which also showed larger superficial areas of inflammation (Fig. 4)) and in the pericardium. The vegetations in the mouth of the inferior vena cava consisted of fibrinoid material with commencing basal organisation. The fibrinous exudates on the pericardium, liver and spleen were also in the early stages of organisation.

Microscopical examination of the two pieces of gum excised during life revealed a complex picture of acute and subacute inflammation. Only very small blood vessels were present. Many of these showed an acute necrosis with thrombosis, hæmorrhage into the surrounding tissue spaces, and dense polymorphonuclear infiltration. Other areas showed a subacute inflammatory reaction with commencing proliferation of fibroblasts. Many eosinophil leucocytes and foreign body giant cells were present.

The skin lesion, 5 mm. in diameter, excised during life from the patellar region, was cut in serial sections. These revealed a central bleb filled with blood. Beneath this the cutis and superficial portion of the subcutis were intensely inflamed and hæmorrhagic. The inflammatory cellular reaction was almost confined to the neighbourhood of the minute vessels in the dermis and consisted almost entirely of neutrophil polymorphs; eosinophil cells were few and giant cells absent. The walls of the vessels were swollen and disintegrated and they were filled with granular debris, desquamated endothelial cells and thrombi. The collagenous fibres of the cutis were separated by hæmorrhage and œdema. The fibres themselves, however, showed no sign of necrosis. No acute fibrinoid lesions were found in any of the arteries of the subcutis. In several of these, however, the intima was eccentrically thickened, possibly as a result of a healed periarteritis nodosa lesion. The thickening consisted of several layers of rounded hollow cells which in sections stained with Weigert's elastic stain, appeared to be embedded in elastic tissue (Fig. 5). The media and adventitia were normal.

Another skin lesion, 24 to 48 hours old, excised from the knuckle after death, presented the same changes in the minute vessels. No abnormalities were noted in the larger vessels.

Case 3.

W.C., a girl aged 17 years, a laundry worker, was admitted to Guy's Hospital on July the 3rd, 1937. She had been in ill health for the past five years. In 1932 her legs became stiff and her feet swollen and very tender. After six weeks in bed she recovered from this attack of "rheumatism." In October, 1934, she suffered from a cough with thick yellow phlegm, associated with attacks of tightness in the chest causing difficulty in breathing in rather than out. This "bronchitis" has continued with exacerbations from time to time, specially in cold weather. Early in December, 1936, pain and stiffness in the legs again became severe, with tenderness and swelling of the ankles and feet. The throat became sore and itchy raised spots appeared on her abdomen and back; these subsided to become large blotchy purple and then brown areas which disappeared slowly. At the end of December, 1936, she developed a colicky pain in the right iliac fossa with diarrhoea and vomiting, and was admitted to the Deal War Memorial Hospital and operated on for appendicitis. The appendix was found to be healthy. After ten days in hospital she returned home feeling better, but two days later acute colicky pains commenced in the left hypogastrium together with severe and bloody diarrhoea. The diarrhoea soon ceased after readmission to hospital. About this time also, she lost the use of her left foot, which also gradually became numb. Since then she has been unable to dorsiflex it; it hangs down and interferes with walking. She was discharged from hospital in February, 1937, and has since not been well, partly because of bronchitis. In June, 1937, the urticarial eruption reappeared on the abdomen and on the feet, together with vomiting and bloody diarrhoea for a few days. The cough became worse and the shooting pains returned in both feet. She had recently lost weight; her periods ceased in December, 1936.

The chief findings on admission to Guy's Hospital were those of peripheral polyneuritis and eosinophilia. The patient was wasted, her weight being 7 stones. The muscular power of both arms was poor, the left being weaker than the right; the grip of the hands was weak and the patient could not adduct the thumb of either hand. Both thenar eminences were obviously wasted. Both legs were thin and the left anterior tibial and peroneal muscles paralysed. In the hands, pain sense was lost over a small area on the right hypothenar eminence and on the palmar and dorsal aspects of the 3rd phalanx of the first and second fingers. Pain sense was lost over the middle third of the lateral aspect of the leg and thigh, and over the posterior and lateral aspect of the calf and over the dorsum, lateral margin outer side of the sole of the left

foot. Sensation to light touch was absent on the dorsum of the foot and toes. Temperature sense was absent from the knee distally. On the right leg, pain sense was lost on the lateral aspect of the calf and lateral margin of the foot; sensation to light touch was impaired over the dorsum of the foot and absent between the first and second toes. The right triceps, biceps and supinator tendon reflexes were absent as were both ankle jerks. A doubtful plantar response was obtained from both feet. The calves of the legs were not tender.

Examination of the blood revealed 5 million red cells, 87% hemoglobin and 21,000 white cells per c. mm.. The red cells showed no abnormality; reticulocytes 3.2%; platelets about 400,000, normal in size. Of the white cells, eosinophils accounted for 39%, neutrophils 47%, lymphocytes 11%, hyalines 3%, basophils present. The anti-streptolysin titre of the blood was found to be 50 units per c.c. of serum. Nothing unusual was found in the cardiovascular system except a simple tachycardia (100 to 120 per min.) out of relation to the mouth temperature (99-100°F.); blood pressure was 121 mm. Hg. systolic and 65 diastolic. The chest showed signs of bronchitis; an X-ray examination of the lungs revealed enlarge dense root shadows and increased striation specially towards the right base. No tubercle bacilli were detected in the mucopurulent sputum which contained a few polymorphonuclear and eosinophil leucocytes. The acid urine (sp. gr. 1020) contained a trace of albumen but no sugar; no red blood cells, pus cells or casts were seen. Examination of the faeces revealed nothing unusual. The skin over the abdomen showed areas of brown discoloration which the patient stated were the remains of the fading rash. The liver and spleen were not felt; some slightly enlarged and firm lymphatic glands were noted in the axillæ and left groin. No abnormality was found in the cerebro-spinal fluid; the Wassermann and Kahn reactions were negative.

The nature of the disease was not at first recognised and it was thought that the skin rash and peripheral neuritis might be due to toxic absorption from a septic focus. The only possible focus found was in the enlarged tonsils and these were enucleated on the 19th July. For a time the patient improved slightly; the low fever subsided and the muscles of the left leg began to recover power. Simple tachycardia (confirmed by electrocardiogram) persisted, the pulse rate being between 100 and 120 beats per min.. The eosinophilia remained high. In the beginning of September, low fever (99-100°F.) again developed, and the patient was observed in a mild but definite asthmatic attack. A few red blood and pus cells were noted in the urine for the first time on September the 10th, and were found on several occasions subsequently. A trace of albumen remained constantly present. Blood urea was 22 mg. per 100 c.c.. On the 13th September the patient complained of vague abdominal discomfort and vomited; the discomfort and fever subsided after a few days and the patient again improved. She now weighed only 6 stone 5 lbs.. In the beginning of October the fever returned. The patient became gravely ill, semi-comatose and incontinent; her pulse rate rose to 140 and respirations to 36 per minute; mild asthmatic attacks aggravated a frequent cough. She was treated in an oxygen tent. After a week the condition improved greatly, but on the 21st October an urticarial eruption appeared on the abdomen and back of the neck. The wheals, about the size of a halfpenny became purpuric and, subsiding, left a reddish and later a brownish stain on the skin. At this time a review of the case in the light of previously published cases led to the diagnosis of periarteritis nodosa. On further examination several small subcutaneous nodules were found on the forearms and legs. One of these was excised from the right forearm and microscopical examination confirmed the clinical diagnosis. The improvement in the patient's condition continued; her weight rose to 6 stone 9 lbs.. No further developments taking place, she was discharged from hospital on the 14th November. A differential leucocyte count then showed only 9% eosinophils, neutrophils 81%, lymphocytes 6% and monocytes 3%.

The patient was readmitted to Guy's Hospital on July the 30th, 1938, because of asthmatic attacks. These had recurred at intervals since she left hospital and had become more frequent during the past six months. She had again lost weight, being now 6 stone 1 lb.. Her periods were now normal. The signs of peripheral neuritis had disappeared, the tachycardia persisted; the blood pressure was 120 mm. Hg. systolic and 70 mm. diastolic. The chest showed signs of bronchitis. The urine was normal. The eosinophils numbered 22% of a total of 6,000 leucocytes. The asthmatic attacks were only partially relieved by adrenaline and the patient was discharged on August the 10th, 1938.

She was admitted to University College Hospital on May the 23rd, 1939, because of asthma and purpuric spots on the extremities, some of which became bullous. The asthmatic attacks were only occasionally relieved by adrenaline. An irregular temperature (up to 99°F.) persisted. The urine showed no signs of nephritis. Blood leucocyte counts revealed a persistent eosinophilia of from 32 to 57%, the total white count being from 10,000 to 15,000 per c. mm.. The patient was discharged from hospital on August the 26th, 1939, just prior to the outbreak of war, and has since remained at home under the care of Dr. Westlake. In November, 1939, he informed me that the patient was comparatively well and free from asthma; in the beginning of 1940 he reported that asthmatic attacks had recurred and usually with her menstrual periods. She attended Guy's Hospital for examination on March the 1st, 1940. Though her general condition had improved she remained thin and pale. Her weight was 7 stone 6 lbs.. The heart rate was

116 beats per minute. One small (2 to 3 mm. diameter) firm nodule was present beneath the skin just below the elbow on the radial side of the right forearm. Hæmoglobin was 82%. The leucocytes numbered 20,400 per c. mm., neutrophils forming 51%, eosinophils 36%, lymphocytes 12% and hyalines 1%.

The excised nodule consisted of vascular adipose tissue with a subacute inflammation specially of the arterial twigs. These showed fibrinoid necrosis of the media and subintimal regions together with a sparse leucocytic infiltration within and around the vessel wall. In several the endothelium was pushed into the lumen by the accumulation of fibrinoid material beneath it, the lumen being narrowed and in places obliterated. One artery, the largest in the nodule, measuring 0.27 mm. in diameter across the media, showed hæmorrhage into the necrotic wall and surrounding tissues (Fig. 7). The infiltrating cells were chiefly neutrophil polymorphonuclears and mononuclears; eosinophils were few and giant cells absent.

Case 4.

H.P., an engineer, aged 38 years, was always healthy and active until the summer of 1937, when he began to suffer from nasal obstruction due to polypi. He attended the out-patient department of Guy's Hospital in July, 1937. A large mass of polypi was removed from each side of the nose in August, 1937. The polypi recurred and were again removed in September, 1938. Further trouble developed and he was admitted to hospital in January, 1939, when bilateral sublabial antrostomy was performed with removal of polypi. After this he became weak, developed a cough and lost weight; he found difficulty in walking because of weakness of his legs. In May, 1939, he again attended the out-patient department, complaining of cough and a severe pain in the chest. His temperature was 99.2°F. and a pleural rub was heard in the left chest. X-ray examination of the chest revealed dense root shadows and calcified glands, but no signs of active disease. No tubercle bacilli were detected in the sputum. His condition was thought to be due to toxic absorption from infected antra and he was re-admitted to hospital in June; nasal polypi were removed and the antra washed out. He continued to attend as an out-patient. He then began to suffer from cold hands and he noticed his finger nails to be blue. His ankles swelled and red spots appeared on the skin over the lower part of both legs. The urine contained a trace of albumen. He was readmitted to hospital on July the 19th, 1939.

The patient was pale and very thin; during his stay in hospital body weight fell from 8 stone 8 lbs. to 7 stone. The lower legs and ankles were cedematous; the cedema soon subsided and did not return. His temperature remained normal throughout but a simple tachycardia persisted, the pulse rate being constantly about 100 per minute. He also suffered from a persistent productive cough for which no adequate explanation was found. A few râles were heard at both lung bases. The lymphatic glands generally, including both epitrochlear glands, were slightly enlarged and firm. The liver and spleen were not palpable.

Repeated blood examination showed a persistent leucocytosis (31,000 to 33,000) due chiefly to a great increase in the number of eosinophil cells; these formed 55 to 65 per cent. of the white cells and were normal in appearance and mature in type; neutrophil polymorphonuclear cells formed 18 to 31 per cent. and lymphocytes 14 to 17 per cent.. The red cells, normal in appearance, numbered about 3½ millions and hæmoglobin, which at first was 75, fell to 66 per cent.. The urine, repeatedly examined, usually but not always contained a trace of albumen, and a few red cells, but no pus or casts.

The heart was normal throughout and blood pressure remained at about 135 mm. Hg. systolic and 90 diastolic. The blood Wassermann and Kahn reactions were negative and blood cultures remained sterile. The anti-streptolysin titre of the blood was found to be 500 units.

For the first few days early morning vomiting occurred; a stomach wash out contained neither mucous nor pus; a test meal revealed hypochlorhydria. On July the 22nd he experienced an attack of bloody diarrhoea; proctoscopy revealed superficial ulceration of the rectal mucosa. The attack soon subsided. A month later blood again appeared in the stool but no rectal ulceration was found to account for it. Initially, the nervous system, central and peripheral, displayed no abnormality. On July the 25th the patient experienced an attack of severe pain in the right arm, the pain shooting down to the fingers. A localised tender spot without induration was found in the biceps muscle; this localised tenderness disappeared within 12 hours but the pain in the arm and hand persisted though diminishing. Signs of median nerve paralysis gradually developed, motor loss appearing first and sensory changes later. Two days later a similar severe pain attacked the right leg and foot but without localised tenderness. This was quickly followed by foot drop and later weakness of the plantar flexors. Sensory loss over the anterolateral aspect of the leg and foot manifested itself a day later and was not an immediate feature. On August the 12th the left leg was similarly attacked by pain followed quickly by paralysis (foot drop and weak plantar flexion) and later by gradually developing sensory loss over the same area as on the right leg. At the end of his stay in hospital, power was beginning to return to the right hand and foot and the anaesthesia was diminishing.

Both hands were generally cold and the finger nails blue. The feet were not affected. The main pulses in the arms, legs, hands and feet were normally palpable. The condition of the hands was partly due to spasm of the vessels; the blueness and coldness largely disappeared when the patient was warm and then the digital pulses were clearly felt at the sides of the proximal phalanges. The reactive hyperæmia test, however, displayed clear evidence of obstruction to small arteries in both hands, specially the left. In the left hand the flush following the release of the circulation appeared immediately in the palm and dorsum and bases of the fingers; it was delayed for 5 seconds in the distal part of the 5th finger, for 20 seconds in the index finger and thumb and for 30 seconds in the middle and ring fingers. In the right hand the flush was delayed for 6 seconds in the tip of the index finger and for 13 seconds in the tip of the 5th finger. There was also a delay of 8 seconds over the central parts of the right thenar and hypothenar eminences; here the flush spread over the pale skin from the periphery inwards. A second test, a week later, gave the same results.

On admission skin lesions were noted on the outer sides of the ankles and over the posterior aspect of the elbows. These consisted of small vesicles, some blood filled and others pustular, and dry crusts, the later stage of the vesicles. The vesicles at the ankles were surrounded by reddened skin, partly due to hæmorrhage. These lesions gradually healed but other isolated vesicles, preceded by red spots, little or not at all tender, developed from time to time on the hands and fingers. On the hands also a few deeper and very tender nodules formed in the palms of the hands and red tender spots under the finger nails like the Osler's nodes of subacute bacterial endocarditis. Two vesicles were excised and examined microscopically, one one day and the other three days old. An inguinal lymphatic gland was also excised.

The combination of symptoms in this case, its general resemblance to that of Case 3 and, in addition the evidence of block of small arteries in the hands strongly indicated the diagnosis of periarteritis nodosa. The first sections cut from the excised tissues, however, failed to confirm the diagnosis. Because of the clinical indication, the remaining pieces were cut in serial section and the characteristic arterial lesions were found. On the outbreak of war, the patient was transferred to the Royal Sussex County Hospital, Brighton, under the care of Dr. Barrington Prowse, who has kindly kept me informed of the progress of the case; I saw the patient there in December, 1939. His condition had improved steadily and considerably. He looked well and could walk with leg splints. His weight had risen to 9 stone 3 lbs.. He remained afebrile. For two months the pulse rate varied between 90 and 100 per minute; since then it had declined to remain at 80 to 90 per minute. The blood pressure was 150 mm. Hg. systolic and 80 diastolic. A troublesome cough had continued but except for bilateral and diffuse medium crepitations, the lungs presented no abnormal physical signs. Except for one slight attack of diarrhoea without blood, on October the 17th and 18th, the bowels had remained normal. Very large pelypi were removed from the nose in October. The skin lesions had rapidly healed and no more developed; glandular enlargement had disappeared. A trace of albumen but no blood was found in the urine from time to time. A blood count on November the 20th revealed 4.8 million normal red cells; hæmoglobin 85%. Leucocytes numbered 12,300 per c. mm., polymorphonuclears 69%, lymphocytes 27% and monocytes 4%. The circulation to the hands had improved but the finger nails still became blue in cold weather. A reactive hyperæmia test gave normal flushing in the right hand (within 3 seconds); in the left hand a delay of 10 seconds in the distal portion of thumb, middle and ring fingers. All the arm reflexes and cutaneous sensation were normal; some wasting of the small muscles persisted but the patient could write. The muscular power of both legs had improved. A large part of the skin covering the extensor muscles and dorsum of the right foot was anæsthetic as was also a small area on the lateral aspect of the distal part of the thigh. On the left leg only a small area on the antero-lateral aspect remained anæsthetic.

Except for the residual palsies the patient remains well (May, 1940).

Microscopical examination of excised tissues. The lymphatic gland contained several areas of necrosis some with marginal hæmorrhage and fibrin deposition, strongly suggesting infarction. The chief artery to the gland was normal but at the hilum many of its branches, over portions of their length and specially at points of branching, showed fibrinoid necrosis of the wall and reduction and obliteration of the lumen by fibrinoid material together with thickening and loosening of the adventitia and inflammatory cellular infiltration. The arteries affected were all very small, their transverse diameter measuring 50 to 70 μ . from outer edge to outer edge of the media. The necrotic lesions appear to be of different ages and the general picture was that of a subacute inflammatory process passing on to healing. The inflammatory cells in the affected areas were mainly eosinophil and mononuclear leucocytes; binuclear and multinuclear giant cells were present. In the older lesions only traces of the original arterial wall could be detected, the affected stretch of vessel being converted into granulation or scar tissue.

The 21 hour old skin lesion, 2 mm. in diameter, consisted of a collection of small foci of collagen necrosis in the dermis surmounted by an epidermal vesicle filled with coagulated albuminous material containing only a few leucocytes, chiefly polymorphs (Fig. 9). The necrotic

fibres were swollen, hyaline and separated; the crevices between them were filled with a faintly granular material staining blue with hematoxylin and infiltrated by polymorphonuclear leucocytes. According to the direction in which bands of fibres were cut, the necrotic areas were rounded or in the form of streaks. The connective tissue surrounding the small vessels between the collagen bands was loosened and contained many inflammatory cells, polymorpho- and mononuclear, and also fibroblasts. Eosinophil cells were not numerous. The walls of the small vessels were swollen but not necrotic. None of the vessels in any of the sections showed the periarteritis nodosa lesion. No bacteria could be detected. The general histological picture was that of collagen necrosis with a subacute inflammatory reaction.

The three day old lesion, 5 mm. in diameter, showed similar changes but the inflammatory reaction was more in evidence and further advanced. The majority of the necrotic foci were surrounded and more or less replaced by young granulation tissue containing many large mononuclear and binuclear cells and some multinucleate giant cells. Eosinophil leucocytes were not numerous. The foci of collagenous necrosis and the surrounding tissue reaction closely resembled the similar foci in the subcutaneous nodules and in the myocardium in cases of acute rheumatism.

At one edge of this lesion a small artery at a point of bifurcation showed fibrinoid necrosis. Only a small part of the original wall was recognisable, the remainder was bulged and replaced by fibrinoid material and granulation tissue containing numerous giant cells while the lumen was almost entirely filled with fibrinoid material. Eosinophil leucocytes were not numerous (Fig. 8).

Case 5.

G.S., an engineer, aged 61, attended the out-patient department of this hospital in May, 1937, because of recurring attacks of pain in the left hand, and of pallor or blueness of the fingers specially in cold weather. Always well and active until about 1930, he then began to suffer from asthma and bronchitis. The first asthmatic attack occurred when he was out walking and he was much troubled with repeated attacks for about a month when they left him entirely. They later returned from time to time, specially during the winter; ephedrine relieved him. In 1933 he suffered for a time from abdominal discomfort; a duodenal ulcer was suspected but not confirmed. Early in 1936 he had an attack of laryngitis. At the end of that year he suffered from pain in the left shoulder, arm and hand. The pain subsided after about 10 weeks, but soon returned and for a time the hand was swollen and tender, chiefly on the radial side. In May, 1937, he experienced two attacks of nettle rash. He had not previously nor since suffered from nettle rash. About this time also he noticed occasional blueness of the fingers (specially the 4th and 5th) and in the palm of the left hand. When blue the fingers were cold and numb, and free from pain; they became red and painful when warmed up. He had never been troubled with his right hand or feet. He had not recently suffered from asthma. Examination showed the left hand to be cold and the tips of all the fingers cyanosed; a blue patch was present on the thenar eminence. The hand and arm were not wasted or lacking in power; sensation was normal. The patient was admitted to hospital for further investigation in June, 1937. Except for the condition of the left hand, a hyperresonant chest and a blood pressure of 160 to 180 mm. Hg. systolic and 80 diastolic, examination revealed no abnormality. His general condition was good. His temperature remained normal and the pulse rate varied between 60 and 90 beats per minute. The superficial pulses were normally palpable. The left hand was usually cold and the tips of the fingers cyanosed, specially the 4th and 5th, which were also tender to touch. The coldness and cyanosis were mainly due to spasm for, when the patient was warmed, all the fingers became normally warm; cyanosis persisted, however, at the tip of the 4th and 5th digits, evidence of obstruction of small arteries. As the spasm was relieved by warming the patient experienced the usual temporary aching and burning of the fingers. In addition, however, he experienced bouts of pain in the left hand, specially in the 4th and 5th fingers at irregular intervals. The bouts decreased in severity and frequency and no adequate explanation was found for them. A week after admission a red patch about 3 cm. in diameter appeared on the skin of the dorsum of the ulnar side of the left forearm; beneath this reddened patch which gradually subsided, a small tender nodule developed. No other similar lesions were found elsewhere. No further developments occurring, the patient was discharged in July, 1937. The pain subsided about a fortnight later. In September, 1937, the terminal phalanges of the 4th and 5th fingers still displayed cyanosis but were no longer tender. The forearm nodule had disappeared. He felt well and, except for a return of abdominal discomfort for a few weeks in October, 1938, remained so until April, 1939. Then while at work, he experienced a sudden sharp pain in the left shoulder; the pain continued and the 3rd and 4th fingers tingled. The next day he was unable to extend these fingers and his wrist was dropped. He attended hospital in May, 1939, with signs of musculospiral palsy. The left arm was thinner than the right and the extensors and inter-ossei were slightly wasted. There was considerable loss of power in the extensor muscles of the arm, forearm and middle finger. Cutaneous sensation was normal. The pain and weakness gradually passed off. In the beginning of July, 1939, the attacks of asthma recurred and were frequent, preventing him working. About the same time also he suffered from an attack of abdominal

pain with distention and general tenderness, but without diarrhoea or vomiting. No cause was found for this and the condition cleared up after a week in bed. In September, 1939, attacks of pain returned in the left hand and fingers. Now, however, he noticed that the hand had become warm and remained warm even when the other hand was cold. He sometimes slept with his hand out of the bed clothes to relieve the uncomfortable warmth. A tender reddened area appeared on the back of the arm above the wrist and another on the palm of the hand between the forefinger and thumb. He attended hospital on several occasions in October and November, 1939. It was at this time that a review of the case led to a diagnosis of periarteritis nodosa. The condition of the muscles of the left hand and arm was unchanged. The hand was warmer than the right except when the patient was warmed. A tender nodule underlying a patch of slightly reddened skin was present on the palm between the forefinger and thumb; a second tender nodule was found at the distal end of the middle phalanx of the middle finger. On the dorsal aspect of the forearm, above the lower end of the ulna, and covering an area 4 cm. long by 2 cm. wide were five small slightly raised, reddened and tender areas of skin overlying small nodules in one of which localised arterial pulsation was detected. This nodule was excised and microscopical examination confirmed the clinical diagnosis. The hand and fingers were not cyanosed and the flush of reactive hyperæmia appeared within three seconds in all parts of the hands and fingers. The other extremities were normal. Apart from the recurring pain in the hand the patient felt well. He was pale (hæmoglobin 65%) but had not lost weight and his general condition remained good. His pulse rate was 72 per minute and blood pressure 145 mm. Hg. systolic and 75 diastolic. The urine was free from albumen and blood. The blood picture was that of a secondary anaemia with lymphocytosis. The leucocytes numbered 15,500 per cubic mm., lymphocytes forming 55%, polymorphonuclears 36%, eosinophils and basophils 2% each and hyalines 5%. By November the 14th, the hand was free from pain and no longer warmer than the right; the nodules, no longer tender, were barely palpable. No other signs of disease were detected.

The patient was last seen in February, 1940. He had continued at work and remained well except for one attack of opistaxis and frequent cramps in the left hand while at work. No signs of disease were detected; all nodules had disappeared. Blood leucocytes numbered 10,300 per c. mm., polymorphs 68%, lymphocytes 22·5%, hyalines 6·5%, eosinophils and basophils each 1·5%.

The excised nodule was cut in serial sections. Examination of these revealed numerous areas of fibrinoid necrosis with a subacute or chronic inflammatory reaction in the small arteries and arterioles in the dermis and subcutaneous tissue. The necrosis affected short stretches of the arteries, chiefly at points of branching and, while in some it involved the whole circumference, in most it affected only a segment of the vessel wall. The fibrinoid material was chiefly subintimal in position and formed cushion like projections covered with endothelium narrowing or obliterating the lumen (Figs. 10 and 11). In some areas, the media was more or less replaced by fibrinoid material. Along the course of the arteries and arterioles the adventitia was considerably thickened and it was this which gave rise to the nodule. The thickening consisted for the most part of a loose myxomatous fibrous tissue in which were many new blood vessels (Fig. 10). In some places the thickening consisted of granulation tissue. Some arteries were more or less completely replaced by granulation or young fibrous tissue in which only traces of the original wall were recognisable and in which one or more new arterial channels were forming. The inflammatory cells present were mainly mononuclear; in a few areas there were small collections of polymorphonuclear leucocytes. Eosinophil leucocytes were sparse and giant cells absent. The veins were not affected. The vascular channels in the dermis were wider than normal and contained lymphocytes and large mononuclear cells. The collagenous tissue of the dermis seemed normal as also the epidermis.

Case 6.

C.M., an engineer, aged 45, was admitted to hospital on August the 17th, 1939, for a rash on the hands and face. He had a long history of attacks of ague, having been infected with malaria in Mesopotamia in 1917 and again on the West Coast of Africa in 1930. At the age of 18 he suffered from "double pneumonia" and since then had been troubled by a chronic cough with expectoration. He had also suffered from stomach trouble; in 1934 he underwent an operation for a perforated duodenal ulcer; he had since experienced mild recurrences of stomach pain. On August the 7th, 1939, an ague attack developed which did not differ from the usual. He stayed in bed for two days and returned to work on the 10th, feeling not quite fit. The next day his throat was sore but he continued at work. On waking on the morning of the 13th he felt a burning of the cheeks and noticed that these and the bridge of the nose were reddened and slightly swollen. During the day, the rash spread to the ears. On the 14th, redness and swelling accompanied by a burning sensation appeared in the palm of the left hand and on the 15th in the palm of the right hand. The next day both hands were generally swollen, red, hot and burning. On the 17th Dr. Barber at the out-patient department diagnosed the condition as erythema multiforme and admitted the patient to the wards. The rash on the face was then subsiding but

both hands to the wrists were generally swollen, red, hot, and tender; on the backs of the hands were scattered many small pustules. The tongue was furred and showed a small apthous ulcer; the gums were slightly inflamed but the fauces and tonsils were normal. The throat was no longer sore. The only other skin lesion was a small slightly raised reddened hot and tender area at the inner side of the left knee. Nothing abnormal was found in the chest or abdomen, or nervous system. The lymph glands were not enlarged. His temperature was 102°F. and pulse rate 100 per minute. Blood sedimentation rate was 60 mm. in one hour. White blood cells numbered 15,500 per c. mm. of which 88% were polymorphonuclear, 1% each eosinophil and basophil leucocytes; 8% were lymphocytes and 2% large mononuclear cells. On the 18th similar red areas appeared on the outer side of the left leg and foot and the patient complained of pain on movement in the right trapezius muscle. No localised tenderness was found there or elsewhere. He complained of a severe headache. On the 19th another patch of reddening was noted on the back of the left thigh and the lower parts of both forearms were reddened. The patient was treated with M. and B. 693 from August the 18th to the 22nd. No further developments took place; no pustules appeared elsewhere than on the hands. By the 21st the redness and swelling had largely subsided and on the 22nd the temperatures and pulse rate fell to normal. X-ray examination revealed a normal stomach but a deformed duodenal cap. A test meal revealed only some delay in the appearance of free hydrochloric acid. No occult blood was found in the stools. The urine contained no albumen, blood or casts. The blood pressure was within normal limits. On August the 25th, when the patient was discharged from hospital, the rash had entirely disappeared and the hands had begun to peel. Throughout this brief illness, his general condition was good. He was a well built short man, then weighing 8 stone 8 lbs.

I saw the patient shortly after his admission. Having in mind the cases of polyarteritis nodosa then under observation, and the history of duodenal ulcer in this case, and being aware that a multiforme erythematous eruption is associated with periarteritis nodosa I considered the possibility of this diagnosis. The possibility was substantiated by biopsy and by the further course of the case. Two pieces of reddened skin were excised, and cut in serial sections, one from the knee, four hours, and one from the thigh sixteen hours after the first appearance of reddening. Both pieces show in addition to acute inflammation in the dermis, fibrinoid necrosis of the arterioles in the subcutaneous tissue and base of the dermis with an inflammatory reaction (Fig. 12). The leucocytes in the necrotic areas were mainly polymorphonuclear; eosinophils were few.

After discharge from hospital the hands peeled completely, no peeling occurred elsewhere. The patient returned to work on September the 4th. In the beginning of November, 1939, he began to feel stiffness on closing the left hand together with some tenderness of the knuckles. At the same time he noticed a small lump in the palm of the hand. I examined him on November the 15th and found five small nodules about 0.5 c.m. in diameter deep in the skin of the palm and one in the web between the thumb and forefinger of the left hand. One nodule was found in the palm of the right hand. No other abnormalities were noted in the skin; there were no signs of peripheral neuritis or of obstruction to the vessels of the hands or feet. The lymph glands were not enlarged. Examination of the chest, heart and abdomen revealed nothing unusual; the pulse rate was 72 per min. and the blood pressure 125 mm. Hg. systolic and 80 mm. diastolic. The urine was dilute (sp. gr. 1002) and contained no albumen, red cells or casts.

The patient was last seen on April the 25th, 1940. He had remained well except for a recurrence of gastric pain during February. The nodules in the palms of the hands were still palpable though smaller; the skin on the dorsum of the hands and fingers was smooth and shiny. The blood pressure measured now 145 mm. Hg. systolic and 95 diastolic; the urine (sp. gr. 1008) contained no albumen, blood, pus or casts. No other abnormality was detected.

Case 7.

M.G., a housewife, aged 43 years, was admitted to hospital on December the 6th, 1937. She had suffered from Bright's disease at the age of 8, and from enteric fever at 17. Further severe kidney trouble developed with her second pregnancy and again, but slight, with her third and last pregnancy. She had for long suffered from frontal headaches; the headaches had recently become more frequent and severe and chiefly occipital in position. Numerous attacks of blurred vision, mainly in the right eye, were also experienced. In the summer of 1937 she suffered from menorrhagia due to a cervical erosion; she was awaiting admission to hospital for a hysterectomy.

Examination revealed chronic nephritis, severe hypertension and renal failure. She passed daily 50 to 60 ounces of urine of specific gravity of 1008, the urine containing a small amount of albumen, some red blood and pus cells and occasionally a few granular casts. The blood urea, initially 145, rose finally to 225 mg. per cent.. The ocular fundi were pale and the arteries severely sclerosed. No abnormality was detected in the nervous system; the Wassermann and Kahn reactions were negative. The heart was enlarged; systolic blood pressure remained

between 210 and 250 mm. and the diastolic between 140 and 170 mm. Hg.. No blood count was made. A moderate tachycardia (pulse rate 90 to 100 per min.) and low irregular fever (temperature 99 to 100°F.) persisted throughout. The headaches and blurred vision continued, she complained of pains in the limbs and became very weak. Her weight fell from 8 stones on admission to 7 stone 4 lbs.. On December the 31st she experienced abdominal pain and the next day much blood appeared in the urine and she became slightly jaundiced. On January the 10th, 1938, she complained of sudden severe pain in the right loin and abdomen which lasted for several hours. She became comatose and died the next day.

At autopsy the peritoneum contained 2½ litres of heavily blood stained fluid, and a large blood clot was attached to the inferior margin of the right lobe of the liver. There was no peritonitis. The liver, otherwise normal, displayed a large subcapsular blood clot on its right lateral and anterior surfaces. The capsule of the liver was ruptured at the right inferior margin where the clot was continuous with that in the peritoneal cavity. It appeared that intra hepatic hæmorrhage had occurred and had ruptured the liver with the production of a diffuse subcapsular hæmorrhage which in turn had ruptured the capsule and made its way into the peritoneum. The right kidney weighed 100 g., was small and completely hydronephrotic. The pelvis and calyces were dilated and surrounded by a thin rim of parenchyma which was pale and devoid of structure. At the pelvi-ureteric junction the ureter was slightly narrowed and its wall thickened; the remainder of the ureter was of normal dimensions. There were no aberrant renal vessels, a single small renal artery entered the kidney at the hilum. The left kidney was large, weighing 250 g.. Its capsule was adherent and its surface showed moderate sized scarring. The parenchyma was uniformly pale but the cortex was slightly and irregularly narrowed and the vascular pattern partially obliterated. The boundary zone was irregular and ill-defined. The pyramids were pale; the pelvis and ureter were normal.

The heart was enlarged, weighing 600 g.; the enlargement was due chiefly to hypertrophy of the left ventricle. The myocardium seemed normal. The aorta and coronary arteries showed slight atheroma. Nothing noteworthy was observed in the respiratory, alimentary, nervous, lymphatic and endocrine systems.

Histological examination revealed widespread changes in the smaller arteries and arterioles. The left kidney contained a few areas of subcapsular ischaemic necrosis with atrophy of some of the glomeruli. A few of the glomeruli also showed patches of fibrinoid necrosis and thickening of the capsule. Many of the arterioles showed severe hyaline degeneration and others fibrinoid necrosis but with little or no inflammatory cellular reaction. The intima of the larger arterial branches was thickened. None of the vascular lesions showed frank inflammation and all could be classed as those usually found in cases displaying malignant hypertension during life. They were not those usually seen in periarteritis nodosa. In the right kidney, however, in addition to these changes the parenchyma was largely replaced by fibrous tissue and larger arterial branches showed numerous acute, subacute and healing lesions characteristic of periarteritis nodosa, with aneurysm formation.

Severe hyaline degeneration was present also in the arterioles of the heart, liver, kidney, spleen, thyroid, adrenals, pancreas, thymus, intestine, pectoral muscle, brain and adventitia of the aorta. Acute, subacute and healing lesions of periarteritis nodosa were present in the arteries and arterioles of heart, liver, pancreas (Fig. 14), intestine, diaphragm and aortic adventitia. These necrotic and inflammatory lesions and those of the right kidney presented no obvious difference from those described in the preceding cases. Many eosinophil leucocytes were among the inflammatory cells; giant cells were not seen. Small aneurysms were found also in the intestine (Fig. 13), pancreas, diaphragm and adventitia of the aorta.

In addition, the myocardium displayed a few scattered foci of inflammation in which the cells were chiefly polymorphonuclear; in the endocardium several small areas of inflammation and hyalinisation of the surface were noted.

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THE ADJUSTMENT OF BLOODFLOW TO THE AFFECTED LIMB IN ARTERIOVENOUS FISTULA.*

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THE following is a brief account of observations upon cases of arteriovenous fistula in the leg resulting from bullet wounds. It is clear that, when the lumens of the main artery and vein of a limb become freely united, much of the blood that should flow to the extremity of the limb is diverted from it, and the circulation to the damaged limb on that account tends to suffer. It tends to suffer in all the limbs for the reason that general arterial pressure is reduced by the leak; there is not the usual driving force to maintain a normal flow through the capillaries. It tends to suffer especially in the affected limb because, while mean arterial in this limb is usually reduced, venous pressure is raised. This tendency for the flow to be especially reduced to the damaged limb becomes counteracted in the course of time, as the following case reports show.

Case 1.

A.V.E., a man of 48 years, was shot in the upper part of the right thigh in August, 1917, and a large arteriovenous fistula resulted. He came first under my observation in February, 1923, and was fully recorded by Drury and myself (6, Case 1). He presented a long pulsating tumour in Scarpa's triangle extending two inches into the abdomen above Poupart's ligament. The man showed conspicuous water-hammer pulse and other signs of leakage from the arterial system, which were largely abolished to the accompaniment of pulse slowing by compressing the right femoral artery. The right common femoral pulse was much larger than the left. The pulses in the arteries of the two feet were all distinctly felt and were not regarded as unequal on the two sides, but the systolic pressure in the right popliteal was 129 mm. and in the left 188 mm.. The right leg was a little larger in circumference (about 1 cm.) than the left. The veins of the right leg were more conspicuous than those of the left, and the pressure in them was about 5 cm. water greater.

* Work undertaken with the aid of the Medical Research Council.

This man was next seen in 1934, eleven years later. His condition was in most respects unaltered; thus the cardiac impulse lay in precisely its former position, 5 cm. beyond the nipple line in the 5th and 6th spaces. But certain changes were noticed, including those which prompt this report. The pulsating, cylindrical tumour in the thigh was changed, it had extended much farther into the abdomen, it had widened, and it was a little tortuous. It was obvious that this tumour was arterial and not venous, for its tension and pulsation were undiminished if the common femoral artery was compressed and closed. His general venous pressure was unraised and he was in good health. The veins of the right leg had become conspicuously varicose and much pigmentation had developed over the leg, especially in relation to swollen veins; a patch of skin over one vein was inflamed. It was noticed that the right leg was warmer than the left from thigh to toes. The man was examined often from this standpoint and the relative hotness of the right leg was repeatedly observed; the toes were sometimes equal in temperature on the two sides, but the right calf was always warmer than the left. The patient had noticed this difference himself. The patient remained under observation.

In 1935, the same difference in the temperature of the legs was noted and it was remarked that the pulses in the right foot were larger than in the left. In November of that year the heat elimination of the two feet was very carefully measured by Stewart's calorimeter, after soaking the two feet in water at 30° for a half hour in a warm room. It was 40% greater from the right than the left foot. General vasodilatation was then induced by immersing the left arm in water at 45°C.. The heat elimination on the right side was doubled, and that on the left side nearly trebled, by this procedure, the elimination on the two sides thus becoming equal. On the same day, both legs being warm, Pachon osillometer readings were repeatedly taken from right and left leg, just below the knee and just above the ankle. The pulse excursion was about twice as great on the affected as on the unaffected side at both levels.

Seen at the end of 1937, in 1938, and in 1939, the right leg continued to be hotter and the pulsation of its arteries greater than on the left side. At no time were palpable anastomotic arteries discovered in the right leg.

Case 2.

C.W., a bus driver of 38 years, came under observation in October, 1935. He was under treatment for duodenal ulcer and for a large intra-thoracic neurofibroma. He had in addition an old-standing arteriovenous fistula.

When 20 years of age he was shot just below the right knee. There was no hæmorrhage at the time and he was without symptoms except for pins and needles in the foot after the wound had healed.

On examination, entrance and exit scars could be seen on the inner and outer side of the right leg just below the knee joint. No tumour could be

felt but the lower part of the popliteal space pulsated unusually and displayed a continuous thrill, maximal in systole. On the right side the external iliac, common and superficial femoral arteries could easily be felt pulsating along the whole of their courses; the corresponding left vessels were much less conspicuous. The external iliac and common femoral on

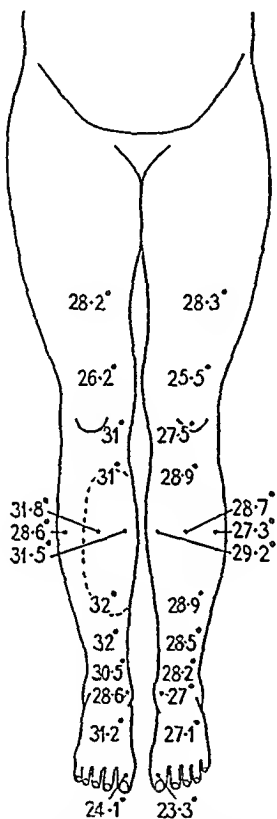


Fig. 1. Caso 2, October, 1935. Arteriovenous fistula in lower right popliteal space. Shows readings of temperature taken by thermoelectric junction from symmetrical points on the legs alternately. The broken outline on the inner side of the right leg was that occupied by palpable tortuous arteries.

the right side seemed considerably dilated. The posterior tibial pulses seemed equal, but the anterior tibial and dorsalis pedis pulses were much stronger on the right than on the left. On the inner side of the right leg, below the knee, tortuous and vigorously pulsating arteries were found and could be traced as low as the middle of the leg, 14 cm. below the level of the wound. The right leg was more flushed than the left and its veins were varicose. The right calf measured 36 cm. and the left 31 cm. in circumference. The right leg was noted on several occasions to be much warmer than the left. A temperature map of the legs after equal exposure for a half hour

in a room at 19° is shown in Fig. 1. Above the ankle, the Pachon oscillometer gave deflections on the right 5 times as great as those on the left side. A difference of equal magnitude was found for the middle of the two legs. As the man lay on his back, the right leg from knee to ankle was perceptibly raised at each beat of the heart.

Heat elimination from the two feet, estimated by Stewart's calorimeter, after soaking both feet in water together at 30° for 25 min., was almost twice as great on the right as on the left side. The left arm was then immersed and maintained in water at 45° , a procedure producing sweating in 10 min.. The heat elimination during the next 15 min. increased only a little in the right foot, while it increased in the left until that of the right foot was reached or a little surpassed.

The heart was slightly enlarged, the upstroke of the pulse was abrupt; the blood pressures in the right arm were 140 systolic and 75 diastolic, the latter rising appreciably, while the pulse slowed, on compressing the right common femoral artery.

Case 3.

A.G. was wounded by shrapnel in July, 1916. The severe wounds were in the right shoulder breaking the scapula, in the right foot, and in the left thigh; there were less serious wounds in the neck and in the face. He was in hospital for 9 months, and afterwards used a crutch, walking on the left leg while his right foot was healing. His chief symptoms were in the right foot but the left leg felt tense, especially when dependent and resting, and the thigh presented a pulsating lump; these last symptoms have remained. He first came under my observation in November, 1938, when he was 45 years of age. He walked without difficulty and complained little. He was a small man presenting large healed scars on the dorsum of the right foot and over the right acromion process; he was rather flushed in face and his skin and mucous membranes showed distinct capillary pulsation. All the superficial arteries pulsated strongly, and the pulse was regular but abrupt in upstroke. The blood pressures in the arm were 150 systolic and 84 diastolic. The veins of the neck were normal and uncongested. There were no signs of cardiac enlargement, and the sounds were normal.

Two small scars were seen in the skin of the lower and inner part of the left thigh. A large swelling extended downwards for 15 cm. from Poupart's ligament to the middle region of the thigh. It was cylindrical, 4 to 5 cm. across, tense, and strongly pulsatile. An enlarged and tortuous external iliac artery joined its uppermost border. Just below its lower border, a little above the scars, and 21 cm. from the middle of the patella, a thrill could be felt maximally. It was continuous but accentuated in systole, extended upwards a little and down Hunter's canal to the top of the popliteal space. The pulsation and thrill ceased when the main artery was compressed at Poupart's ligament. The same compression lowered the pulse rate from 72 to 60, and raised the systolic pressure from 150 to 157 and the diastolic

pressure from 84 to 100 mm. Hg.. Pulsation of the popliteal arteries was easily felt, the right being the stronger. The right popliteal systolic pressure was 153 and the left 140 mm.. The posterior tibial and dorsalis pedis pulses were easily felt in both feet and were not noticeably larger on one side than the other. The left calf was 3 cm. more in circumference than the right

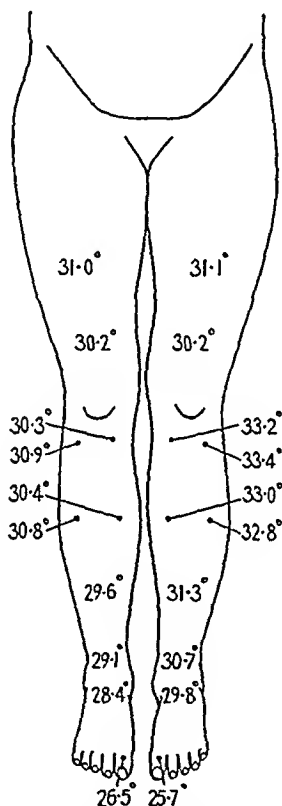


Fig. 2. Case 3, November, 1938. Arteriovenous fistula in middle of left thigh. Readings of temperature taken by thermoelectric couple from symmetrical parts on the legs alternately are shown.

and much the tenser. Pachon's oscillometer gave much larger pulsations below the knee and at the calf on the left than on the right side. The veins of the left leg were much more conspicuous than those of the right, were very tense and a little tortuous. The skin of the whole left leg from the knee down was more deeply flushed than the right, and presented remarkable patches densely covered with pigment spots.

On the many occasions on which he was examined warm, or after exposure of the legs, the left leg from knee to near the ankle was always much warmer than the right, by as much as 3°C.. The patient himself knew of this difference, and it is illustrated by the readings of the accompanying

chart (Fig. 2). The differences in the temperatures of right and left foot were inconspicuous, sometimes the right (as in Fig. 2) but more often the left were slightly the higher. Careful search revealed no palpable collateral arteries. The heat elimination of the two feet, estimated under exactly similar conditions to those previously described, did not differ greatly, the left foot showing a slight excess, both before and after heating the patient until he sweated freely.

Previous records. A search of past records has discovered a number of relevant cases previously published.

A case of arteriovenous fistula of the femoral vessels consequent upon a knife wound received 7 months earlier was recorded as early as 1833 by Breschet (2). An attempt to relieve the condition surgically led to gangrene of the leg and death of the patient a week later. At the post-mortem examination the arteries of the affected leg were found to be dilated and tortuous even as far as the foot.

Later Broca (3) referred to occasional cases of ligation of arteries, for example ligation of the femoral artery for popliteal aneurysm, in which the temperature of the affected limb distally was subsequently found to be higher than that of the normal limb. He also said that limbs affected by arteriovenous aneurysms are hotter than normal. He explained the fact as the result of congestion of the skin capillaries, itself due to obstruction of venous return. He ventured to express the curious view that the blood passes from the vein into the artery during its diastole.

Franz (4) described a femoral arteriovenous fistula in a boy of 14 years, caused by a knife wound 18 months previously. The leg was $2\frac{1}{2}$ cm. longer, and its calf $1.5^{\circ}\text{C}.$ hotter than on the unaffected side, though the pulse in the foot was scarcely perceptible. Resection of the sac showed the peripheral and proximal artery to be of similar size. He ascribed the increased length of leg to increased blood supply, noting a similar event in osteomyelitis. He was aware that most authors describe the distal parts of the affected limb as colder than normal.

Holman (5) described a popliteal arteriovenous fistula in a man of 41 resulting from a bullet wound received 25 years before; he speaks of the foot and leg being as much as $2^{\circ}\text{F}.$ hotter on the affected side, and says that the patient was aware of this increased warmth. Later in discussing clinical records and his experimental observations he is content to ascribe the dilatation of the artery leading to the fistula, and of arteries in its neighbourhood, to the distending force of the greater bulk of blood attracted to the part; distal dilatation occurs in his view when, through obstruction of the lumen proximal to the fistula, distal channels form the avenue of approach for the large quantity of blood attracted to the fistula.

Reid (9) described the case of a man shot in the thigh 9 months before, with an arteriovenous fistula just below Poupart's ligament. The foot on the affected side was colder but the leg from the ankle upwards was warmer than on the normal side. He also described a man of 35 years, wounded in

the left subclavian vessels 5 months before with resultant arteriovenous fistula. Although the systolic pressure was much higher in the right arm, the left forearm was hotter than the right. His observations led him to believe that an abundant collateral circulation may develop around an arteriovenous fistula. He refers to experiments by Zinniger on dogs as showing a more extensive development of collateral circulation around an A-V fistula than around a ligated artery. In emphasising the powerful stimulus which the fistula provides for the development of the collateral channels, he says that when we consider that the parts peripheral to a fistula are deprived of their allotted blood supply by reason of the shunt, there is little wonder that nature makes a prodigious effort to compensate by collateral channels.

Discussion.

It seems clear that, although the establishment of an arteriovenous fistula, acting as a short circuit, at first decreases the blood supply to the distal parts of the limb, this state is not maintained. With passage of time the bloodflow to the distal parts tends to become restored. The recovery may in fact proceed to the point where there is actually a greater flow than to the normal limb.

This increase in circulation as time proceeds may be due in some measure to a reduction of vasomotor tone in the damaged leg. Evidence for this was found in Cases 1 and 2, for heating the body induced a greater vasodilatation in the undamaged leg. But this was not found in Case 3 and could not be regarded as adequately explaining the first two cases. For if compensatory vasomotor relaxation were the sole cause of the increased flow to the affected limb, then, under general vasodilatation, such as was induced, the normal should become more fully supplied than the affected limbs, since flow to the affected limbs must be impaired by the increased pressure in the veins of these. Thus it may be inferred that there is a more persistent widening than can be ascribed to simple loss of vasomotor tone. This inference is obviously supported by the facts. It is generally recognised that the artery leading to an arteriovenous fistula becomes greatly enlarged. Though less generally recognised, it has I think been proved that there may be vascular growth distal to the fistula. I may instance the appearance of palpably tortuous arteries on the inner side of the damaged leg below the knee in Case 2. The collateral channels in this case might conceivably have developed out of obliteration of the posterior tibial artery below the level of the anastomosis; but the evidence is against the presence of such an obstruction, a full posterior tibial pulse being found at the ankle. In Breschet's case already cited, the enlarged tortuous anastomoses were found on dissection actually to extend to the ankle. Thus there is evidence that in cases of arteriovenous fistula the defect in distal circulation may be compensated not by simple dilatation but largely by arterial growth. Such

a conclusion is very significant to present views of the formation of collateral circulation.

When a main artery to a limb becomes blocked, certain factors come into play, at once or after very short delay, and tend to restore the circulation to the distal tissue. As Recklinghausen (8) pointed out there is, as a result of the obstruction, a little rise of pressure proximal to it, while there is a decided fall of pressure in the artery and its branches distal to it. These immediate changes in pressure increase the flow in branches issuing from the main artery proximal to the obstruction and supplying the territory in which the pressure is lowered. Moreover, as Bier (1) concluded, the vessels of the deprived territory will enter a state of reactive hyperæmia, owing as we should now say to the accumulation of vasodilator substances in the corresponding tissues.

These early adjustments help to restore circulation. They are, however, inadequate to explain the formation of permanently enlarged channels. Thoma (10) concluded on the basis of many observations that such growth of collateral channels occurs because bloodflow through them is increased. In this he was supported by Nothnagel (7), who also expressed the view that the vascular growth is due to increased nutrition consequent upon increased flow through the vessel affected, or consequent upon increased flow through its vasavasorum. It is to be noted that such collateral anastomoses develop whether the limb is previously deprived of nerves or not; the development is independent of nerve supply. Thoma's theory, that the growth or shrinkage of arteries is controlled by the amount of blood flowing through them, might be held adequately to explain the development of collateral channels when the main artery of a limb is obstructed. It might be held to explain the well recognised increase in the size of the main artery leading to an arteriovenous anastomosis. But it will not explain what is here reported, namely, an increase in the size of arterial channels very distal to a simple fistula; for the fall in arterial and rise in venous pressure, which are the necessary consequences of the original lesion, must tend primarily to retard rather than to increase bloodflow in these channels. It is clear that hydrostatic factors provide no common basis to explain the compensatory growth of collateral vessels, around an arterial block and the growth of vessels distal to a simple arteriovenous fistula. Searching for a common factor we find ourselves returning to Reid's generalisation, that the arterial channels develop to meet the needs of tissue deprived in part of blood supply. Chief interest lies in enquiring how this can come about. Neither adjustment of nervous control, nor a direct response of the affected vessels to increased pressure or nutrition, can be regarded as a satisfactory explanation; and we are brought to ask if arterial growth is not directly controlled by a stimulant, a chemical stimulant arising locally as a product of the tissue need, and acting locally. The growth of collateral channels is so locally adjusted and occurs under such different circumstances of pressure and flow, that it now seems quite necessary to formulate an intimate and special

mechanism to explain this permanent increase in size. The essence of the matter seems to be that there is a local call by tissues in need and that to this call there is a local and adequate response.

Summary.

1. Cases of arteriovenous fistula of the limbs are described in which the circulation to the distal part of the limb becomes restored to, or actually beyond, normal.

2. This compensatory increase appears to develop gradually over a period of years.

3. It may be due in part to decreased vasomotor tone, but may also be due to vascular growth.

4. The growth of the distal arterial supply in arteriovenous fistula is of great interest from the general standpoint of collateral circulations. The idea that this growth may be explained simply by altered pressures or altered bloodflow such as follow in the affected vessels as an immediate consequence of the original arterial lesion, cannot be supported. It is suggested that the growth is controlled by a stimulant, a chemical stimulant, arising locally as a product of the tissue need and acting locally.

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PITUITARY LIKE FACTORS IN THE BLOOD AND URINE OF DIABETIC PATIENTS AND OF ANIMALS TREATED WITH PITUITARY EXTRACTS.*

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THE work initiated by Houssay has definitely established that factors from the anterior lobe of the pituitary gland exert a profound influence upon the carbohydrate metabolism of normal and depancreatised animals (10). It has been shown that the anterior pituitary gland is responsible for the degree of diabetes mellitus which appears after pancreatectomy and further that extracts of this gland can produce a diabetic state in normal animals. These findings have naturally stimulated clinical workers to search for the presence of anterior pituitary like factors in human cases of diabetes mellitus. So far this search has produced no conclusive evidence that any such factors are present in these cases nor even that if they were present they could be demonstrated. Nevertheless, the volume of physiological work on the anterior pituitary gland continues to grow and to suggest more and more strongly the possibility that factors from the anterior pituitary gland may play an important part in the mechanism of human diabetes mellitus, and that a search for such factors in the patient's body fluids might prove successful. It is, therefore, necessary that investigations of this possibility should be continued. The investigations reported in this paper had as their object the demonstration of the presence or absence of anterior pituitary-like factors in the body fluids of normal men and animals, of diabetic patients and of animals rendered temporarily "diabetic" by injection of anterior pituitary extracts. Unfortunately the programme of work has been interrupted and the number of experiments in certain investigations are insufficient to allow the drawing of final conclusions. We believe, however, that the tentative conclusions in these curtailed investigations will be found substantially correct by those who have opportunity to complete the work.

Two factors of the anterior pituitary gland have been sought; a factor which antagonises insulin action, the "glycotropic factor" (17), and a factor which promotes accumulation of fat in the liver, the "liver fat

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increasing factor" (1). Owing to the fact that many substances on injection into normal animals interfere with the action of insulin in depressing the blood sugar it is necessary here to state precisely the characteristics of the anti-insulin effect, which we have accepted as presumptive evidence of the presence of an anterior pituitary like factor. Different preparations of the anterior pituitary gland may on injection into the normal animal produce three types of effect in relation to insulin action. First they may produce a rapid rise of the blood sugar level associated with a greater or less degree of retardation or curtailing of insulin action. Second they may produce, after a latent period, a temporary diabetic state characterised by hyperglycæmia, glycosuria, ketonuria and marked inhibition of insulin action. Such preparations on repeated injection may give rise to a permanently diabetic state, and it is to these extracts that Young restricts the term "diabetogenic" (18). Thirdly the preparation may have no effect upon the blood sugar level yet completely inhibit the action of insulin. Young has called the factor giving rise to this third effect, the glycotropic factor (17) (18) (19). The first effect is non-specific. We do not mean to imply by this statement that a particular factor capable of causing a relatively rapid rise of the blood sugar level does not occur in the anterior pituitary gland, and that the several reports of such a substance in the urine of diabetic patients are of no account (5) (11) (13) (16); we simply wish to state that this property of causing an immediate rise of the blood sugar level is common to extracts of many tissues (18) and that therefore its demonstration in an extract is not presumptive evidence of the presence of an anterior pituitary like factor. We have found, not infrequently, that extracts of normal urine may possess this property. This objection does not apply to the effects either of the diabetogenic or glycotropic factors each of which has properties peculiar to itself. Tests for the diabetogenic factor were impracticable since its demonstration requires quantities far larger than are likely to be found in the blood and urine of diabetic patients. The glycotropic factor on the other hand produces its effects in relatively small amounts. It is characterised by its ability to produce, some hours after its injection into normal animals, a state in which insulin action is inhibited whilst the blood sugar level remains normal (17). These characteristics are peculiar to this factor and not easily counterfeited. In searching for evidence of an anti-insulin factor in the body fluids we have therefore sought for the characteristics of the glycotropic factor.

The presence of the glycotropic and "liver fat" factors has been sought in blood and urine. The general plan of investigation has been as follows. First a reliable technique for detecting the factors when mixed with body fluids has been selected and tested. Second, donor animals have been injected with preparations of these factors in amounts required to produce maximal effects and then the presence of the factor has been sought in the animal's body fluids. Third, the methods for detecting the factors have been applied to the body fluids of diabetic patients.

METHODS.

Pituitary extract used for injecting donor animals (18). Fresh ox pituitary glands were obtained direct from the slaughter house. Immediately after removal from the animal they were frozen by contact with carbon dioxide snow and on their arrival at the laboratory the anterior lobe was dissected from the posterior lobe. All the anterior lobes from one batch of glands were weighed, ground with sand in ice cold saline, and the mixture, after adjusting to pH 8.5, allowed to stand overnight in the ice chest. Next morning the mixture was readjusted to pH 8.5, centrifuged at 3000 mins R.P.M. for 15 min., the supernatant liquid separated and the volume of the fluid adjusted so that 3 c.c. corresponded to 1 g. fresh anterior lobe. This crude pituitary preparation was used on the day of preparation and, whilst awaiting use, was kept in the ice chest at 2°C.

In all experiments in which evidence was sought of the transmission of pituitary like factors by the body fluids of donor animals to recipient animals, control animals in the recipient group were injected with known amounts of the crude pituitary extract in order to test both its potency and the sensitivity to the extract of the particular batch of animals. All injections of pituitary extracts were given subcutaneously.

The test for the glycotropic factor. The test depends on the comparison of the rate and extent to which a standard dose of insulin, injected intravenously, depresses the fasting blood sugar level before and after injection of the preparation suspected to contain this factor.

Male rabbits, approximately 2 k. in weight, were used. For at least one week before testing they were given a standard diet of cabbage, oats and bran. Eighteen hours before the test food was withdrawn. At 9 a.m. next morning the rabbits were brought to the laboratory and warmed near an electric heater. Three hours later they were placed in a box, warmed by a hot water bottle, and remained in the box throughout the test. Samples of blood for estimation of sugar were obtained from one lateral vein of the ear and 0.5 unit of insulin injected into the corresponding vein of the opposite ear. Three samples were taken before injection of insulin and after the injection samples at 5, 10, 20, 30 min. and thereafter at 15 min. intervals up to 2½ hr.. The curve obtained is called the insulin depression curve. A control curve was first obtained from each animal. Three or four days later a second curve was obtained after injection of the preparation under investigation, and in some animals a further control curve was carried out a week after this. At 6 p.m. on the day prior to the test of the preparation half the amount of preparation was injected subcutaneously and at 9 a.m. on the morning of the test the other half was similarly injected. The insulin was injected intravenously three hours after this second dose.

The blood sugar was estimated in 0.1 c.c. of whole blood by the Hagedorn-Jensen method.

The lower limits of sensitivity of this test have not yet been determined, but it was found that two injections of our crude extract of pituitary gland each corresponding to 250 mg. of the fresh gland inhibit the action of 0.5 unit of insulin.

The test for the liver fat factor. This test depends upon the property of a factor present in the pituitary gland to increase the amount of fat in the liver of fasting mice (1) (2).

Female white mice, all of the same stock, three to five weeks old and weighing 15-17 g. were used. For one week prior to the experiment they were given a diet of rat-cake (15) containing 7% of fat. Before the test the mice were separated into groups of 10, each group being of the same weight. Food was then withdrawn and the control solution or the extract to be tested was injected subcutaneously. The injection was made about 5 p.m. and the animals killed by stunning and then cutting their throats, 17-18 hours later. The livers were rapidly dissected out and the 10 livers of each group weighed together and analysed. The fat content was estimated by the method of Kumagawa and Soto (14). The results are expressed as g. fat per 100 g. of body weight (2).

Fasting alone is known to cause some increase of liver fat in mice and therefore in all experiments a control group of 10 mice from the same batch of animals was injected with a volume of normal saline equal to the volume of the preparation injected into the animals (2). In most of the experiments another group of 10 mice was injected with the same crude pituitary extract as had been injected into the donor animals from whose body fluid the preparation under test had been prepared. Presence of the liver fat factor was revealed by a significant increase in the fat content of the livers of the animals injected with the preparation under test above that found in the control animals.

By means of this test the amount of the liver fat factor contained in 5 mg. of fresh anterior pituitary gland can be certainly detected (2). (Table I).

Concentration of the glycotropic and the liver-fat factors from urine. The method employed was that of Greep (6) which was successfully used by Cope (3) for the recovery of the pituitary thyrotropic principle from urine. Briefly the method is as follows. The urine is made alkaline to litmus and 20 g. of sodium benzoate dissolved in each litre of urine. The urine is then made acid to congo red by addition of hydrochloric acid and the resulting precipitate filtered and sucked dry on Buchner filter. Extraction of the dry precipitate with acetone leaves a small amount of insoluble residue which contains the active principle. This is collected in a Buchner funnel and washed with acetone and ether. The dry powder thus obtained can apparently be kept for a considerable time without losing potency. It is prepared for injection immediately before use by dissolving in $\frac{N}{20}$ sodium hydroxide and then neutralising the solution to litmus.

When this procedure is applied to the urine either of normal men or of normal rabbits a final residue is obtained, but this possesses no detectable glycotropic or liver fat increasing effect. It is worthy of note, however, that occasionally and inconstantly residues obtained from the urine of both normal and diabetic subjects may, on injection into rabbits, raise the fasting blood sugar level.

Glycotropic factor added to urine can be satisfactorily recovered by this method. To 8 litres of the pooled urines of young normal subjects 12 c.c. of a crude pituitary preparation, equivalent to 4 g. of fresh anterior pituitary gland, were added. After standing for 3 hours at room temperature, the urine was extracted by the sodium benzoate method. The extract was injected into a rabbit and completely inhibited the effect of the insulin on the blood sugar. We have not had the opportunity as yet of determining if, by the benzoate method, glycotropic factor in lower concentrations than that corresponding to 500 mg. of fresh anterior pituitary gland per litre urine can be recovered satisfactorily.

The liver fat factor added to urine can be recovered apparently completely by the benzoate method. Crude pituitary preparation corresponding to 50 mg., 150 mg., and 300 mg. of fresh anterior pituitary gland respectively was added to three separate litre volumes of urine. These three volumes and a further litre of urine to which no pituitary preparation had been added were separately extracted by the benzoate method. Each of the resulting four extracts were tested on a group of 10 mice, each animal of which received an equal amount of extract, so that the amount of extract received by each animal in each of the four groups corresponded respectively to 0 mg., 5 mg., 15 mg., or 30 mg. of the added anterior pituitary gland. Four further groups of 10 mice were taken. The first of these groups received injection of saline; each animal of the remaining three groups received respectively amounts of crude pituitary preparation equivalent to 5 mg., 15 mg., and 30 mg. of fresh anterior pituitary gland. From Table I which summarises two such series of experiments (A and B) it will be seen that the increase in liver fat of each group of mice receiving corresponding amounts of either the preparation or of the extract was similar.

The urine from diabetic patients and healthy subjects was collected in bottles, which contained a few grammes of sodium benzoate. Whilst awaiting extraction the bottles were kept in a cool room. The bottles were brought to the laboratory twice a day and immediately extracted. Recent experiments on rabbit's urine suggest that better preservation of anterior pituitary like principles is obtained if the urine is collected in bottles kept cold by means of ice.

Concentration of the liver fat factor from blood. Coagulation of the blood was prevented by addition of sodium citrate, the red cells were removed by centrifuging and the tests carried out with the separated plasma or an extract made from it. The blood from human patients was obtained from the antecubital vein: the blood from the rabbits from the lateral ear vein

if small quantities were required, from the carotid artery, after anæsthetising, if large quantities were needed.

TABLE I.

Table showing that the liver fat increasing factor can be reclaimed apparently completely from mixtures of urine with preparations of the anterior pituitary gland.

No. of mice.	Injection per mouse.	Liver fat g. 100 g. body weight.	Increase of liver fat.	No. of mice.	Injection per mouse.	Liver fat g. 100 g. body weight.	Increase of liver fat.
10 (A)	Saline	0.481		10 (A)	Extract of 100 c.c. normal urine.	0.342	
10 (A)	Saline + equiv. of 5 mg. Ant. Pit.	0.626	0.145	10 (A)	Extract of mixture 100 c.c. urine + equiv. of 5 mg. Ant. Pit.	0.506	0.164
10 (A)	Saline + equiv. of 15 mg. Ant. Pit.	0.880	0.399	10 (A)	Extract of mixture 100 c.c. urine + equiv. of 15 mg. Ant. Pit.	0.800	0.458
9 (A)	Saline + equiv. of 30 mg. Ant. Pit.	1.075	0.594	10 (A)	Extract of mixture 100 c.c. urine + equiv. of 30 mg. Ant. Pit.	0.918	0.576
10 (B)	Saline.	0.434		10 (B)	Extract of 100 c.c. normal urine.	0.392	
				10 (B)	Extract of mixture 100 c.c. urine + equiv. of 10 mg. Ant. Pit.	0.694	0.302
10 (B)	Saline + equiv. of 20 mg. Ant. Pit.	0.744	0.310	10 (B)	Extract of mixture 100 c.c. urine + equiv. of 20 mg. Ant. Pit.	0.671	0.279
				9 (B)	Extract of mixture of 100 c.c. urine + equiv. of 50 mg. Ant. Pit.	0.850	0.468

Plasma from the rabbits injected with crude preparations of the anterior pituitary gland, whilst perfectly satisfactory for injection into other rabbits in testing for glycotropic activity, is not satisfactory for direct injection into mice in the test for the presence of the liver fat factor. The plasma from the injected rabbits shows a marked lipæmia and the amount of this blood fat is sufficient in itself to cause a significant increase of liver fat in the injected mice. A method of extraction was therefore sought which would not only concentrate any active principle present, but which would also ensure a fat free extract. The method described by Hewitt (7) for the

precipitation of plasma proteins was finally found to satisfy these requirements.

After withdrawal of the blood, the plasma was separated immediately and chilled to 0°C.. Subsequent procedures were carried out in a cold room at 0°C.. The plasma was sprayed, from a syringe through a fine hypodermic needle, into a mixture of absolute alcohol and dry ether at a temperature of 15°C.. The alcohol and ether were mixed in the proportions of 7 : 3 and sixty volumes of this mixture were used for each volume of plasma. After standing for two hours the precipitated protein was separated by filtration through a pleated filter paper, washed with the alcohol ether mixture, then with dry ether and finally dried in vacuo. At no time until the precipitated proteins were thoroughly dry were they exposed to a temperature higher than 0°C.. The dry powder thus obtained apparently keeps indefinitely at room temperature. It is made up for injection by dissolving in normal saline. This method can also be applied to crude pituitary preparations without causing any apparent loss of activity either as regards the glycotropic factor or the liver fat factor. When such an extract from normal plasma is injected into mice a slight increase of the liver fat is usually obtained. Whilst this increase is insufficient to raise the possibility that the plasma of normal animals contains a specific factor raising the liver fat it is sufficient to render it advisable to inject a control group of animals with an extract of normal plasma when testing for the liver fat increasing factor.

This method of extraction was only used when the plasma was to be tested for the liver fat factor. The completeness with which this factor can be reclaimed by the method is shown in the following experiment. A crude preparation of the anterior pituitary gland was added to normal rabbit plasma and an extract prepared from the plasma by the above method. Six groups of ten mice were taken. The mice of the first group were each injected with saline, each of those of the second group with the crude preparation of the anterior pituitary gland equivalent to 10 mg. of the fresh gland. The mice of the other four groups were each injected with the extract of the mixture of plasma and crude pituitary preparation which contained the equivalent of 10 mg. of fresh pituitary gland. The increase in liver fat was 164 mg. per 100 g. of body weight in the mice injected with the crude pituitary preparation, 251, 285, 234 and 136 mg. per 100 g. of body weight in the four groups of mice injected with the extract. Unfortunately the injection of a control group of mice with an extract of normal plasma was omitted in the experiments of this group as its advisability had not then been appreciated. Opportunity to remedy this omission later was denied by circumstances. We do not, however, think that the error thus introduced materially affects the conclusion to be drawn from these experiments.

The glycotropic factor in blood. No attempt was made to extract this factor from plasma for the following two reasons. First, lipæmia is of no importance in the anti-insulin test ; second, the rabbit is a sufficiently large animal to receive the volume of untreated plasma it was desired to inject.

RESULTS.

Resistance of anterior pituitary factors to incubation with body fluids and tissues.

The object of the present investigation was to test for the presence or absence of anterior pituitary like factors in blood and in urine and, although the preceding account of the methods of extraction will have made clear that it is possible to reclaim added pituitary factors from artificial mixtures of crude pituitary preparations with these fluids, the possibility still remains that deterioration of these factors may occur when solutions of them in body fluids are subjected to the physical conditions existing within the body. It was therefore an essential preliminary to the present work to determine the effect on pituitary preparations of incubation at body temperature with body fluids and tissues.

Submaximal doses of a crude pituitary preparation were incubated at 37°C. for 3 hrs with whole blood, citrated plasma and serum. No detectable diminution of the liver fat increasing action was found. Equally without effect was the incubation at the same temperature and for the same time of similar amounts of the preparation with a brei of muscle or of liver. A similar investigation was made with regard to the effect on the glycotropic factor of incubation with whole blood at 37°C. for 3 hrs. No deleterious effect was noted but these experiments are not as definite as those on the liver fat factor, because amounts of extract greater than the minimum required to produce a detectable effect were used.

The only observation made on the effect of urine upon anterior pituitary preparations was that these preparations suffered no deterioration when mixed in submaximal doses with urine and allowed to stand at room temperature for several hours. The routine of cold storage and frequent collection of urine we adopted warrants the assumption that any pituitary like factors excreted in urine would be extracted before any significant deterioration had occurred.

It appears therefore that the liver fat factor and the glycotropic factor, in the form in which they exist in crude pituitary preparations, are stable in the presence of body fluids at body temperature. This conclusion does not, of course, exclude the possibility that these factors exist in the body in a form different from that in which they are present in the crude pituitary preparations, and that in this form they may be more susceptible to the body fluids.

THE GLYCOTROPIC (ANTI-INSULIN) FACTOR.

I.—*Investigations for the presence of the glycotropic factor in blood.*

(a) *Investigation of the blood of rabbits made completely insensitive to insulin by injection of crude anterior pituitary preparations.* Six rabbits were injected with two doses of a crude anterior pituitary preparation each equivalent to 9 g. of fresh anterior pituitary gland. This dose was 36 times the dose of the particular preparation required to inhibit completely the

action of 0.5 unit of insulin on the fasting blood sugar level. Eighteen hours after the first injection and three hours after the second each animal was bled out, the blood centrifuged immediately and the separated plasma injected in two equal doses at these same time intervals into another rabbit. The total amount of plasma injected into each recipient animal varied from 38 c.c. to 50 c.c.. Three hours after the second injection of plasma 0.5 unit of insulin was given intravenously and the insulin depression curve determined. In five out of the six recipient animals no trace of glycotropic effect was found. In one there was a moderate anti-insulin effect but we consider that this can be discounted as the animal was found to have a large abscess. This result is in accord with the previous work of Houssay and Foglia (12) who showed that replacement of the blood of a normal dog by the blood of an animal rendered temporarily diabetic by injection of an anterior pituitary preparation did not produce insensitivity to insulin in the recipient animal.

(b) *Investigation of the blood of diabetic patients.* Twenty seven diabetic patients were investigated. All had diabetes of such severity as to require insulin. Of these five were young with diabetes of acute onset and were therefore presumably of the insulin sensitive type. The remainder were either proved to be insulin insensitive by the insulin-glucose test (8) (9) or were of the type with obesity and hypertension typically associated with insensitivity to insulin. The blood of seven of the patients was tested individually on rabbits, the blood of the remaining twenty patients was divided into three "pools." The first pool comprised the blood of the five sensitive patients, the second the blood of six insensitive patients, the third that of nine insensitive patients. The amount of plasma injected into the test rabbits varied from 12 c.c. to 69 c.c., save in one case only in which the amount injected was 5 c.c.. In order to obtain a higher concentration of plasma in the test rabbits animals weighing only 1 to 1.5 k. were used.

In only two out of the twenty-seven patients was any anti-insulin effect discovered and in each of these cases there are reasonable grounds for considering the result an artefact. In one case 5 c.c. of plasma produced inhibition of insulin in the test rabbit. In this experiment the blood was slightly hæmolyzed and on repeating the test on another rabbit with another sample of the patient's plasma untainted by hæmolysis no anti-insulin effect was found. In the other case the injection of the plasma into the rabbit was followed by profuse hæmaturia. We have thus been unable to confirm previous claims (4) that the blood of certain diabetic patients on injection into rabbits has an anti-insulin effect.*

* Similar investigations have recently been published by other workers. Dohan (*Proc. Soc. exp. Biol. Med.*, 1938, 39, 24) tested the serum of 34 diabetic patients but found no significant anti-insulin effect; Marble, Fernald and Smith (*Endocrinology*, 1940, 26, 735) tested the plasma of 30 diabetics and although they found an anti-insulin effect in two samples, further samples from the same patients on injection into different test animals showed no effect. Rushton (*Proc. staff meetings of Mayo Clinic*, 1940, 15, 417) has reported recently an anti-insulin effect in the plasma of 6 out of 27 diabetics but he gives few details and no evidence that the effect observed was significant.

(c) *Investigation of the blood of a case of hyperpituitarism.* The patient was a classical case of Cushing's syndrome associated with diabetes mellitus of sufficient severity to require 25 units of insulin daily for its control. The diabetes mellitus, as judged by the insulin-glucose test, was of the insensitive type. The opinion that pituitary overfunction was either responsible for, or participated largely in, the production of the patient's illness rests on the observation that after irradiation of the pituitary region, the patient's hypertension disappeared, her periods returned, she ceased to require insulin and became sugar free for the first time since her illness was discovered.

The patient was bled and 120 c.c. of plasma obtained. Two doses each of 40 c.c. were injected into one rabbit and two doses of 20 c.c. into another. The first rabbit received as a test dose 0.5 unit of insulin, the second 0.25 unit. No trace of glycotropic effect was found in either animal.

II.—*Investigations for the presence of the glycotropic factor in urine.*

(a) *Investigation of the urine of rabbits made completely insensitive to insulin by injection of crude pituitary preparations.* In these experiments crude anterior pituitary preparations were injected into donor rabbits, the urine from these animals was extracted by the benzoate method and the extract injected into recipient rabbits on whom insulin depression curves had been performed previously.

The early experiments in the series were occupied mainly with solving certain difficulties that arose in connection with obtaining satisfactory urine specimens from the donor rabbits. Crude anterior pituitary preparations have a strong anti-diuretic action and in order to obtain adequate amounts of urine this action had to be overcome. This was achieved by giving daily 60 c.c. of a 10% solution of urea by stomach tube. The pituitary preparations were injected subcutaneously twice daily. In the early experiments the total dose was given within 24 hours, but this was found to be unsatisfactory mainly because of the inadequate amount of urine obtained. The final and satisfactory plan which was adopted was to divide the total amount into 6 equal doses, one of which was injected on the evening of the first day, one on the morning and one on the evening of the second and third days and one on the morning of the fourth day. The total amount of urine passed over this period of 72 hours averaged 500 c.c.. The collection of urine over this long period made it advisable to take precautions against deterioration. The urine was led from the metabolism cages, in which were the donor animals, to bottles surrounded by crushed ice. Each day the bottles were emptied and the urine extracted immediately by the benzoate process. The technique used finally can best be illustrated by the following experiment.

Over a period of 72 hours, at the times stated above, the donor rabbit received injections of crude anterior pituitary preparation each equivalent to 3 g. of fresh gland, i.e., it received a total amount of preparation equivalent

to 18 g. of fresh gland. Throughout the experiment the animal remained in a metabolism cage and received the stock diet of cabbage, oats and bran. Urea solution was given daily and the urine was collected in ice cold bottles. The total amount of urine obtained was 500 c.c.. This was extracted by the benzoate process, the extract dissolved in $\frac{N}{20}$ sodium hydroxide and given as two equal injections to the recipient rabbit 18 hr and 3 hr respectively before the test insulin depression curve. The extract produced practically complete inhibition of insulin action. The extract did not raise the recipient rabbits fasting blood sugar level but it did produce a fatty liver (liver fat 3.06% of the wet weight). The effects therefore of this benzoate extract were similar to those of Young's glycotropic factor.

Circumstances allowed only three experiments to be carried out by this technique but all three gave unequivocally positive results. In the period during which this technique was being evolved many experiments on the urines of rabbits injected with anterior pituitary preparations were carried out and inconstantly, but with increasing frequency as the technique improved, positive results were obtained. Extracts of normal rabbit urine have invariably shown no glycotropic activity. We feel therefore that our experiments, although fewer in number than is desirable, indicate that, after injection of crude anterior preparations into rabbits, a pituitary like anti-insulin factor is excreted into the urine.

(b) *Investigation of the urine of diabetic patients.* Extracts of the urine of diabetic patients by the benzoate process were injected into rabbits and the sensitivity of these animals to insulin tested. The quantities of urine extracted varied from 4 to 10 litres in different patients but in each case the bulk of extract obtained was not too great to inject into single test rabbits in the usual two injections. Extracts from similar quantities of normal human urine showed no glycotropic effect.

The urine from four elderly diabetics, clinically of the insulin insensitive type and all requiring insulin, was tested in this way and no trace of glycotropic effect was found. This number of cases is, of course, quite insufficient to permit definite conclusions but the significance of these few negative results is enhanced by the positive results recorded in the next section.

(c) *Investigation of the urine of cases of hyperpituitarism with diabetes mellitus.* The urine of three cases of hyperpituitarism was investigated in the manner described above. Two of the cases at the time of testing did not show clinically obvious diabetes mellitus but a diabetic type of blood sugar tolerance curve was obtained from each and both showed definite insensitivity to insulin. The third case had frank diabetes.

The first case was an acromegalic, aged 39 years. In the first test 9430 c.c. of urine were extracted and the extract injected in the usual two doses into one rabbit. Complete insensitivity to the action of 0.5 unit of insulin injected intravenously was produced. In addition this extract

produced in the rabbit marked lipæmia and an elevation of the fasting blood sugar level to 204 mg./100 c.c.. Twenty-four hours later lipæmia was still present and the fasting blood sugar level was 208 mg./100 c.c.. The plasma fatty acids were 2210 mg./100 c.c., plasma cholesterol 202 mg./100 c.c. and the liver fat content 4.2% of the wet weight. Following this test the patient was given a course of X-ray irradiation to the pituitary gland and at the end of this the test was repeated. 8880 c.c. of urine were extracted and the extract produced in the test rabbit definite but incomplete inhibition of insulin action (Fig.). On this second occasion, however, the extract produced neither lipæmia nor elevation of the resting blood sugar level and the liver fat content was only 1.4% of the wet weight.

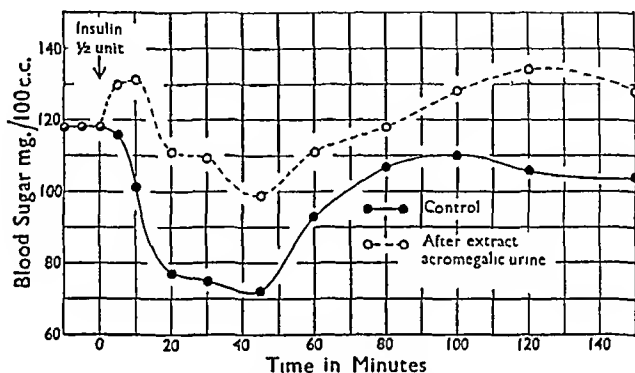


Fig. 1. Two insulin depression curves obtained on the same rabbit after injection of $\frac{1}{2}$ unit of insulin. The control curve is distinguished by discs and a continuous line. The curve following the previous injections of the benzoate extract of an acromegalic's urine is distinguished by circles and a broken line. The fasting blood sugar level on both occasions was 118 mg./100 c.c.. The test shows partial inhibition of insulin action. The extract used was the one obtained from the acromegalic patient after treatment (see text).

It will be seen that the effect of the extract on the second occasion was similar to that produced by submaximal doses of the glycotropic factor. On the first occasion, however, there was not only inhibition of insulin action but elevation of the fasting blood sugar level, marked lipæmia and increase of liver fat. Whilst benzoate extracts of normal urine occasionally produce a similar but less persistent elevation of the blood sugar and some degree of inhibition of insulin action they do not, in our experience, produce lipæmia, fatty infiltration of the liver and complete inhibition of insulin action. These effects, however, are produced by heavy doses of crude saline extracts of the anterior pituitary gland whilst smaller doses produce only a glycotropic effect. We therefore suggest that the effect of the first extract of this patient's urine could be attributed to the presence of large quantities of anterior pituitary like principles and we are strengthened in this opinion by the observation that after treatment, when the urine might be expected to contain smaller amounts of these principles, the benzoate extract of urine

showed a weaker inhibitory effect on insulin similar to that produced by the glycotropic factor and by small doses of crude pituitary preparations.

The second patient was the case of Cushing's syndrome previously mentioned, but the present test was not carried out until after the course of X-ray irradiation and the only remaining evidence of diabetes was the elevated blood sugar tolerance curve and an insulin-insensitive response to the insulin-glucose test (9). 7690 c.c. of urine were extracted and the extract produced a slight but, in our opinion, significant inhibition of insulin action. The extract produced neither lipæmia nor elevation of the resting blood sugar level in the test animal.

A similar result was obtained with urine from the third case of Cushing's syndrome with definite diabetes mellitus.

These extracts are the only extracts of human urine we have met in which there has been any sign of glycotropic activity.

THE LIVER FAT INCREASING FACTOR.

I.—*Investigations for the presence of the "liver fat increasing factor" in blood.*

(a) *Investigation of the blood of rabbits given large injections of a crude anterior pituitary preparation.* Donor rabbits were injected with two injections of a crude pituitary preparation each equivalent either to 9 g. or 10.6 g. of fresh anterior pituitary gland. Three hours after the last injection they were bled out and the plasma proteins separated by the cold alcohol ether method of Hewitt (7). The plasma protein, after dissolving in a small quantity of saline was injected into groups of mice. At the same time blood from normal rabbits was treated in the same way and the proteins from it injected into control groups of mice. This control is necessary as injection of plasma proteins themselves produce in many animals an increase of liver fat greater than that produced by injection of saline.

The results of two experiments on two separate batches of mice are shown in Table II. It will be seen that excluding the group 1b no significant difference is shown between the fat contents of the livers of mice injected with control plasma extract or those injected with the plasma extract from pituitary treated animals. In group 1b the liver fat value for the control group of mice, 0.234 g., is unusually low. The corresponding liver fat value in the mice injected with the plasma extract from the pituitary treated rabbits is at 0.414 g., about the usual value (cf. Table I). We are therefore inclined to regard the difference between the values in these particular two groups not as indicating that the plasma extract from the pituitary treated rabbit contained any of the liver fat factor but that for some unknown reason the fat content of the liver in the control group of mice was subnormal. We consider therefore that our experiments provide no evidence for the presence of the liver fat factor in the blood of rabbits injected with crude anterior pituitary preparations, but before a definite conclusion can be reached on this point further experiments are desirable.

TABLE II.

Table showing that the effect of plasma from normal or pituitary injected animals on the liver fat of mice factor.

Batch.	Plasma extract from control rabbits.			Plasma extract from pituitary treated rabbits.		
	No. of mice in group.	Volume of plasma extracted per mouse.	Liver fat g. per 100 g. body wt.	No. of mice in group.	Volume of plasma extracted per mouse.	Liver fat g. per 100 g. body wt.
I	7	4 c.c.	0.355	8	4 c.c.	0.378
	8	8 c.c.	0.234	8	8 c.c.	0.414
II	7	4.5 c.c.	0.496	10	7.5 c.c.	0.463

The alcohol ether method of extraction was used because by means of it the lipæmia could be completely removed from the plasma of the donor rabbits. In 15 experiments similar to the above, but in which methods of extraction were used which did not render the plasma completely free of fat, no increase of liver fat was found that could not be accounted for by the fat injected in the extract.

(b) *Investigation of the blood of diabetic patients.* Not done.

(c) *Investigation of the blood of cases of hyperpituitarism.* Not done.

II.—Investigations for the presence of the liver fat increasing factor in urine.

(a) *Investigation of the urine of rabbits given large injections of a crude anterior pituitary preparation.* In the technique finally adopted for this group of experiments the donor rabbits were treated in exactly the same way as in the experiments designed to detect the glycotropic factor in the urine of rabbits injected with a crude preparation of the anterior pituitary gland. The urine was extracted by the benzoate method and the extract, after dissolving in $\frac{N}{20}$ sodium hydroxide, was injected in equal doses into a group of mice. A control experiment was also made with an extract from normal rabbit's urine.

The experiments in this group are not conclusive. All gave an indication that the urine extract from the pituitary treated rabbits produced a greater deposition of fat in the mouse's liver than did the extract from the urine of normal rabbits, but in only one experiment was this increase of such a degree as to suggest the presence of a significant quantity of the liver fat factor. This experiment was in many ways the most satisfactory in that an adequate amount of urine was obtained by giving urea and this urine was collected in bottles surrounded by ice. In this experiment the control animals showed 0.372 g. of liver fat per 100 g. body weight and the mice injected with the urine from the pituitary treated animals 0.594 g. of liver fat per 100 g. of

body weight, an increase of 60%. This increase is of about the same order as would be produced by an injection of a crude pituitary preparation containing the equivalent of 5 mg. of fresh anterior pituitary gland.

There is here the first hint of a discrepancy. In all these experiments the maximum amount of the liver fat factor it is permissible to suspect is the equivalent of an injection of 5 mg. of fresh gland per mouse, or, as there were ten mice in the group, a total excretion equivalent to 50 mg. of the fresh gland. In similar experiments, in which the extract from approximately the same volumes of urine was tested for anti-insulin activity on rabbits, complete inhibition of insulin action was found. We think that the degree of insulin inhibition observed in those experiments would not have been achieved by injecting less than the equivalent of 200 mg. to 300 mg. of fresh pituitary gland and we therefore suspect that the glycotropic factor escapes into the urine more easily than the liver fat increasing factor.

Our general conclusion with regard to these experiments is that whilst more careful attention to technique, especially in producing an adequate diuresis in the donor rabbits and in collecting the urine at low temperature, may reveal the presence of small quantities of the liver fat increasing factor in the urine, yet it is unlikely that this factor is excreted in easily appreciable quantities.

(b) *Investigation of the urine of diabetic patients.* Eleven benzoate extracts were made from the urines of ten diabetic patients. Two of the diabetics were clinically of the insulin-sensitive type and the remainder of the insensitive type. One of the sensitive patients was, when the urine was obtained, in advanced ketosis on the verge of coma. The volume of urine extracted varied from 450 c.c. to 4550 c.c., averaging 2200 c.c.. No trace of a liver fat increasing factor was found in any urine.

(c) *Investigation of the urine of a case of acromegaly.* The benzoate extract from the urine of the case of acromegaly referred to previously was tested for liver fat increasing factor. 2700 c.c. of urine were extracted. No trace of a liver fat increasing factor was found. A similar benzoate extract of the urine made at the same time showed marked anti-insulin activity. This discrepancy supports the suggestion made previously that the glycotropic factor escapes into the urine more easily than the liver fat increasing factor.

SUMMARY.

(1) Techniques are described for detecting in blood and urine small quantities of the glycotropic (anti-insulin) factor and the "liver fat increasing factor" of the anterior pituitary gland.

(2) No destruction of the glycotropic factor occurs if crude extracts of fresh anterior pituitary gland containing it are incubated with whole blood at 37°C. for 3 hours.

No destruction of the "liver fat increasing factor" occurs if crude extracts of fresh anterior pituitary gland are incubated at 37°C. for 3 hours with whole blood, citrated plasma, serum or with a brei of muscle or of liver.

Mixtures of crude extracts of the anterior pituitary gland with alkaline urine allowed to stand for several hours at room temperature do not deteriorate either in anti-insulin or liver fat increasing activity.

(3) (a) No trace of anti-insulin activity was found in the plasma of rabbits rendered completely insensitive to insulin by injecting large amounts of a crude anterior pituitary extract, nor in the plasma of diabetic patients, nor in the plasma of two cases of hyperpituitarism with diabetes mellitus.

(b) Definite anti-insulin activity was found in the urine of rabbits injected with crude pituitary extracts and in the urine of the three cases of hyperpituitarism with diabetes. No anti-insulin activity was found in the urine of ordinary cases of diabetes mellitus.

(4) (a) No evidence of the liver fat increasing factor was found in the plasma of rabbits injected with large quantities of a crude preparation of the anterior pituitary gland.

(b) No trace of the liver fat increasing factor was found in the urines of cases of diabetes mellitus or in the urine of a case of acromegaly. Tests of the urine of rabbits injected with crude pituitary extracts are inconclusive and do not exclude the possibility that small quantities of the liver fat increasing factor are present in the urine of such animals.

(5) There is some indication that the glycotropic factor escapes more easily into the urine than does the liver fat increasing factor. If this be the case then estimation of the urinary excretion of glycotropic factor should prove a profitable investigation in patients suspected of secreting excessive amounts of those anterior pituitary factors concerned with carbohydrate metabolism.

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SOMATIC SIMULATING VISCERAL PAIN.*

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IN recent papers Lewis and I (1, 2) showed that the deep tissues give rise to but a single type of pain which may be produced by stimulating either visceral or somatic structures, and that the deep tenderness, skin tenderness and muscular rigidity commonly associated with visceral disease can all be provoked by stimulating appropriate interspinous ligaments. From this work it became clear that painful conditions of the interspinous ligaments or other deep somatic structures in the trunk would give rise to a clinical picture resembling that of visceral disease, and the purpose of this paper is to report examples of a number of cases in which this has been found to be so.

The patients investigated came to hospital because they complained of pain which was thought to be arising from visceral disease, but a careful examination of the somatic structures as well as the viscera, suggested that this pain was of somatic origin. This was confirmed by the fact that the pain could be reproduced by manipulating the suspected muscle or ligament, and that anaesthetising this structure with 2 per cent. novocaine abolished the symptoms completely.

The following is a selected series of cases in which pain was severe, and illustrating the main observations.

Case 1. A soldier of forty years who was invalided for "heart disease." For four months he had suffered from pain in the front of the chest and down the outer side of the left arm to the elbow. The pain first came on when he was driving a heavy lorry and was associated with a fainting attack. It had been diagnosed angina pectoris. Latterly, the pain had been continuous, with exacerbations not clearly related to exercise, except when using the left or both arms. He had also been somewhat short of breath.

He was a healthy muscular man. There were no signs of organic cardio-vascular disease, and exercising the patient produced no increase of pain. The movements of the left shoulder were limited and painful, particularly those involving the pectoral muscles. A tender spot was found

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† Post Memorial Fellow.

in the left pectoral muscles and 20 c.c. of novocaine injected at this point gave complete relief.

X-ray of left shoulder showed no abnormality.

Case 2. A business man of sixty years who had retired two years previously because he had been told that he suffered from "myocarditis." He had suffered from increasing pain in the chest for five years and some shortness of breath. The pain was increased by walking and other exercise, but did not disappear entirely at rest, there being a continuous slight ache.

He was a healthy elderly man, with a barrel shaped chest. There were no signs of cardio-vascular disease. Touching his toes ten times gave considerable increase of pain but little rise in pulse rate. The spine showed an upper dorsal kyphosis and forced flexion and extension of this part gave severe pain.

Pressure over T3 interspinous ligament and to the left of T4 and 5 spines produced severe pain. 10 c.c. of novocaine injected into this region abolished the spontaneous pain, and it could no longer be provoked by exercise.

X-ray of spine showed senile kyphosis (3) maximal in upper thoracic region.

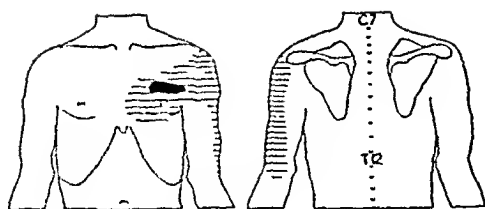
Case 3. A housewife of 48 years. For 1 year she had suffered from attacks of epigastric pain lasting for several weeks at a time, with free periods of a month or two. The pain became worse 1 hour after meals, and was sometimes associated with nausea, but she never vomited. She obtained relief by taking alkali, though this never abolished the pain which was continuously present day and night. She had a similar attack of pain 4 years previously.

A gastric ulcer was suspected, but a barium meal showed no abnormality.

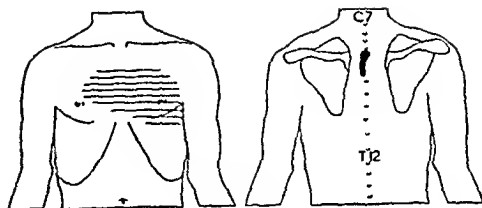
She was a placid well nourished woman. A tender spot was found in the epigastrium just to the left of the mid-line, but no abdominal rigidity and no palpable mass. The spine was kyphotic in the mid-dorsal region, and forced extension of this part gave severe pain in the epigastrium. Pressure over T8 interspinous ligament, and to the left of T8 and 9 spines was painful. 10 c.c. of novocaine injected into this region abolished all pain, and the epigastric tenderness.

X-ray of the spine showed senile kyphosis maximal in the mid thoracic region.

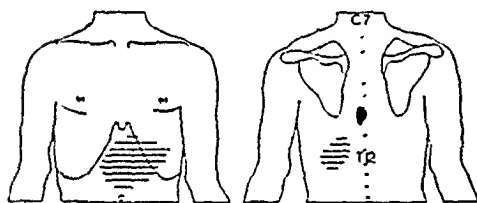
Case 4. A housewife of sixty years who for six months had suffered from pain felt in front over the right costal margin, and over the lower angle of the right scapula behind. The pain was continuous, but she had severe attacks lasting from ten to fifteen minutes. These were associated with nausea and flatulence, but she had never vomited or been jaundiced. Cholecystitis was suggested, but a cholecystogram had shown no abnormality.



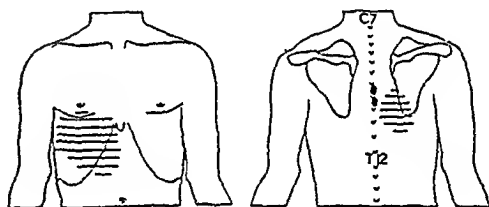
Case 1.



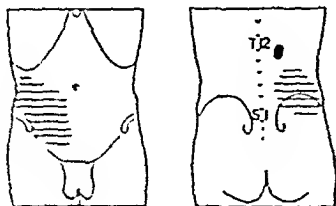
Case 2.



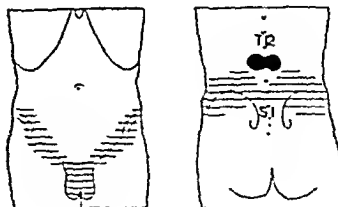
Case 3.



Case 4.



Case 5.



Case 6.

Fig. 1. Shows the distribution of pain (hatching) and its point of origin (black) in the first 6 cases reported in the text.

She was a stout pale woman. There was some tenderness and rigidity of the upper abdomen below the right costal margin but no tumour could be felt. The spine showed a mid dorsal kyphosis, and forced flexion and extension of this part gave severe pain. Pressure over T6 and 7 interspinous ligaments was painful. 10 c.c. of novocaine injected in this region abolished the pain together with the tenderness and rigidity of the abdomen.

X-ray. Spine shows senile kyphosis maximal in mid thoracic region.

Case 5. A housewife of forty-two years who was sent up to hospital as a case of acute appendicitis. On the previous day she felt a sudden pain in the right iliac fossa when lifting a heavy chair. Since then she had been in continuous pain. She had vomited four times and still felt very sick.

She looked healthy though obviously in considerable pain. The pulse and temperature were normal, and the tongue clean and moist. There was considerable tenderness and some rigidity over the right iliac fossa, but no tumour could be felt in the abdomen. The back movements were all very limited and painful, and pressure over a spot in the right erector spinæ at the level of the 12th thoracic spine was painful. 10 c.c. of novocaine injected in this region abolished the pain, together with the tenderness and rigidity in the abdomen, and the back movements became full and painless.

X-ray of spine showed no abnormality.

Case 6. A meat porter of fifty-eight years who had suffered from pain in the lower back and loins for two years. This was a constant ache, but he had severe attacks in which the pain extended into his groins and testes. He had some frequency of micturition and occasional stiffness of his back. Though suspected of renal or ureteral pain, a full urological examination had failed to reveal any abnormality.

He was a thin wiry man. There was no tenderness or rigidity of the abdomen and neither kidney was palpable. Both testes were very tender, but otherwise felt normal. The spine showed a lower dorsal kyphosis, and the back movements were limited and painful, forced extension giving pain in the groins and testes. Pressure on either side of L1 spine was painful and 30 c.c. of novocaine injected in this region abolished all pain and testicular tenderness.

X-ray of spine shows localised kyphosis, probably the result of an old compression fracture of the 1st lumbar body.

The following three cases illustrate respectively, visceral simulating somatic disease (*Case 7*); somatic pain occurring in a patient with visceral disease (*Case 8*), and symptoms probably the result of a combination of both visceral and somatic disease (*Case 9*).

Case 7. A timber porter of fifty-three years. For three weeks he had suffered from pain felt in the chest and left arm. The pain came on suddenly while he was lowering an unusually large piece of timber. Since then he has had a slight ache which became severe on exertion and on use of the arm.

He was a healthy muscular man. No signs of cardiovascular disease could be detected, and the back and shoulder movements were full, but stretching the left pectoral muscles produced pain. There was skin tenderness over the left side of the chest and down the inner side of the arm to the elbow. A very tender spot was found over the left pectoral muscles, but 20 c.c. of novocaine injected into this region altered the pain but little, and failed to prevent the occurrence of a typical anginal attack when he touched his toes twenty times. His subsequent history continued to suggest coronary disease as the correct diagnosis.

Case 8. A housewife of sixty-five years. For three months she had suffered from pain in the left side of the chest. This was constantly present, but she had attacks of very severe pain lasting several hours. These attacks occurred mostly at night, and were quite unrelated to exercise. She had also been short of breath for several years.

She was a stout elderly woman, obviously in great pain. The neck veins were not congested, but the heart was enlarged, the blood pressure 205/110 mm. Hg. and an electrocardiogram showed bundle branch block. Some skin tenderness was found over the left breast.

She had an upper dorsal kyphosis and flexing or extending the spine gave very severe pain in the chest. Pressure to the left of the 5th thoracic spine was painful. 10 c.c. of novocaine injected at this point abolished the pain completely though the skin tenderness persisted for some hours.

X-ray of spine shows advanced senile kyphosis maximal in upper thoracic region.

Case 9. A foreman of thirty-one years. For eighteen months he had suffered from pain in the lower back, and more recently it had extended to the groins and testes. The pain was brought on by using his back, and was clearly related to posture. He had also suffered from frequency of micturition for many years. Ten years previously he fell 18 feet and injured his back.

He was a healthy looking muscular man. A clinical examination of the urogenital tract revealed nothing abnormal, except some tenderness of the testes. The spine was kyphotic in the lumbar region and the back movements were limited and painful. Pressure on either side of the 1st lumbar spine was painful, and 20 c.c. of novocaine injected into this region relieved the pain and testicular tenderness but did not abolish either completely.

X-ray of spine showed narrowing of upper lumbar discs with prolapse of disc substance into the vertebral bodies.

On several occasions the urine contained much pus, and a pyelogram showed dilatation of both renal pelves.

Discussion.

From these cases it is clear that pain arising from somatic structures may closely simulate that of visceral disease. The resemblance is often remarkable. Not only is the character and distribution of the pain identical, but it may be associated with other "visceral" symptoms and signs. For instance, somatic pain in the chest simulating angina may be associated with breathlessness (*Cases 1 and 2*) and the test of exercise may even be misleading as in *Case 2*, in which exercise produced a characteristic increase of pain, and it was not until the back lesion had been anaesthetised that the patient could exercise freely, so demonstrating the somatic origin of the symptoms.* Similarly, abdominal pain may be associated with nausea (*Cases 3, 4 and 5*) and even vomiting (*Case 5*), and the flatulence of cholecystitis (*Case 4*) and the frequency of renal disease (*Case 6*) may also be reproduced. Most remarkable was the epigastric pain of *Case 3*, which was regularly increased one hour after meals, and this increase was relieved by taking alkali, so that it is not surprising that a gastric ulcer was suspected. When, in addition we find "visceral" signs such as the appropriate abdominal tenderness (*Cases 3, 4 and 5*) and abdominal rigidity (*Cases 4 and 5*) the clinical pictures become almost indistinguishable.

Somatic disease might be expected to give rise to pain on movement, but in many of these cases the normal back movements were painless, and it was not until the spine was examined segment by segment that pain could be provoked. Pain on movement may also be misleading as it is often present in conditions such as a retrocaecal appendix or perinephric abscess. It may therefore be very difficult to decide whether a given pain is of visceral or somatic origin.

In such cases it is obviously desirable to make a positive diagnosis of somatic disease rather than a negative one by exclusion of visceral disease, and for this purpose the test of local anaesthesia may be invaluable. This test is, however, not quite as simple as it sounds. It is not always easy to define the source of pain; the finding of tender spots in the back may be misleading owing to the presence of referred tenderness, so that a more certain guide is a careful examination of all the back movements in the relevant segment of the spine, and one should endeavour to reproduce the full pain by a given manipulation. Unless the pain is severe at the time of examination it is rarely possible to get a conclusive result.

Certain clinical features may, however, be helpful. If the pain is continuously present day and night for long periods it is probably somatic in origin, while visceral pain more frequently comes in attacks. The finding of a localised kyphosis in the appropriate segment of the spine is of considerable value, as this deformity frequently gives rise to pain of segmental

* It is instructive to compare this case with *Case 7* in which anaesthetising the tender spot in the pectorals did not alter the severity of the anginal pain provoked by exercise.

distribution. Such a deformity should always be searched for as it is easily overlooked.

By a careful consideration of these points, and the test of local anaesthesia, many cases of obscure suspected "visceral" pain may be elucidated, but in spite of this there are still cases of pain in which as yet no satisfactory conclusion can be reached.

SUMMARY.

(1) Six illustrative cases are recorded in which pain of somatic origin closely simulated that of visceral disease, such as angina pectoris, gastric ulcer, and renal or biliary disease.

(2) Three cases are recorded illustrating :—(a) visceral simulating somatic pain. (b) somatic pain occurring in a patient with visceral disease. (c) a case in which both somatic and visceral disease contributed to the symptoms.

(3) The clinical diagnosis of such cases is discussed.

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A SIMPLIFICATION OF THE EVANS BLUE METHOD OF BLOOD VOLUME DETERMINATION.

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Two methods are available for the clinical study of blood volume, the earbon monoxide method of Haldane, and the dye injection method first made applicable by Keith, Rowntree and Geraghty (7). The weaknesses of the dye method are due to the natural pigments of serum, to its opalescence and to the heavy clouds of fat which are present after meals. Many workers in the United States and elsewhere have shown that the natural colour and opacity of serum prevent ordinary colorimetric estimation of admixed dye (5). Accurate measurements are only obtainable by the use of a monochromatic spectrophotometer (2), an expensive instrument not everywhere obtainable. Even with this instrument, measurements are impossible in the presence of lipæmia, so that cases for study have to be maintained on special diets.

All these difficulties would be solved by extraction of the dye into clear solution free from other pigments. Such a process has now been devised for a dye whose other properties are favourable, and has been tested practically by estimating the blood volume of fifteen normal men and women. Although the method described below is almost certainly susceptible of improvement, it is felt that the procedure offers advantages of simplicity and practicability which make its publication desirable in present circumstances.

Choice of a suitable dye. Many dyes can be used for the estimation of plasma volume (1). Evans blue (T. 1824) has already been widely adopted. It is stable in solution for many months, and can be sterilised by autoclaving. The colour is well suited to colorimetric or other optical forms of estimation. In rats, this dye is non-toxic in dosage over one hundred times as great, weight for weight, as that needed in man. Over a hundred human cases have been injected by other workers, in doses of 0.2 mg. per kg., with no ill effects (2, 9) and our fifteen cases with similar doses have had no reaction of any kind. This dosage produces no general coloration of skin or sclera,

* Working on behalf of the Medical Research Council.

† Working on behalf of the Medical Research Council Committee on Traumatic Shock.

and we have found that any staining produced by accidental subcutaneous injection fades within fourteen days. Evans blue disappears from the bloodstream very slowly, and this property is important for several reasons. Firstly, all dye methods of estimating blood volume depend on calculating from subsequent readings the probable dilution at the time of injection, if mixing in the bloodstream were instantaneous. Secondly, if the natural loss of dye is very gradual, changes in blood volume some hours after the injection of the dye can be deduced from changes in dye concentration. It is therefore clear that Evans blue has much to recommend it for blood volume estimations, provided that its determination in plasma can be improved.

Extraction of T. 1824 from plasma.

Principle. Evans blue is an azo dye having both amino and sulphonic acid groups in its molecule. The sulphonic acid groups are present in greatly preponderating amount and it is therefore to be expected that the dye will be extractable from aqueous solution into a partially miscible organic solvent such as a higher alcohol if the pH is so low that all the acidic groups are undissociated. Experiment shows in fact that the dye begins to pass from aqueous solution into *n*-butyl alcohol when the pH is lowered to about 2.5; from strongly acidified aqueous solutions the transference to butyl alcohol becomes quantitative, whilst at pH 3.5 and above the alcohol fails to extract any significant amount of dye.

Now the pigments and lipid substances in normal plasma which interfere with the direct determination of the dye are mostly neutral or weakly acidic in character, and are readily soluble in butyl alcohol. It should therefore be possible, by preliminary extraction of a weakly acidified mixture of dye and plasma with butyl alcohol, to remove these interfering substances; addition of strong acid to the aqueous phase followed by a further extraction should then yield a butyl alcoholic solution of the dye in relatively pure condition.

Actually direct treatment of plasma containing the dye in this manner is impracticable owing to the massive precipitation of denatured proteins which is caused by agitation of plasma with butyl alcohol (*see* Graff and Clarke (4)). We have found, however, that this difficulty can be overcome if the plasma-dye mixture is first submitted to a brief digestion with pepsin; the small precipitate which forms when such digested plasma is shaken with butyl alcohol interferes neither with the removal of plasma pigments and lipins nor with the subsequent quantitative extraction of the dye; it is this observation which forms the basis of the method to be described.

Method. Solutions:—

10% aqueous solution of pepsin (Parke Davis 1:10,000) freshly made up.

5 N Hydrochloric acid.

5 N Sodium hydroxide.

n-Butyl alcohol, aldehyde-free (prepared by shaking commercial *n*-butyl alcohol with saturated aqueous sodium hydrogen sulphite for 8-10 hrs, drying the butyl alcohol layer over anhydrous potassium carbonate and fractionating).

Butyl alcohol, saturated with 0.85% sodium chloride solution by shaking in a separating funnel.

5 c.c. of plasma containing dyo are placed in a 20 c.c. centrifuge tube and to it are added 0.5 c.c. N HCl, giving a pH of about 1.7, and 1 c.c. of 10% pepsin solution. The mixture is shaken, then incubated for three hours in a water bath at $40 \pm 2^\circ\text{C}$. The solution is adjusted to about pH 3.5 by adding 0.35 c.c. 5 N NaOH from a micro-burette. It is then shaken vigorously with 2.5 c.c. of butyl alcohol and centrifuged for 5 to 10 minutes at 3,500 r.p.m.. Three layers are formed: an upper one of butyl alcohol containing plasma pigment and lipins, a thin disc of precipitate coloured with the dye and a lower aqueous layer containing the rest of the dye. The supernatant butyl alcohol layer is removed with a capillary pipette attached to a filter pump and the washing process is repeated twice more. After the third quantity of butyl alcohol has been removed as completely as possible, 4 c.c. of 5 N HCl are mixed with the residual solution and 5 c.c. of the butyl alcohol saturated with saline are added. The tube is again vigorously shaken and centrifuged, with a cap to prevent evaporation. The butyl alcohol layer now contains the dye practically free from other pigments, whilst the precipitate and lower aqueous layer are quite colourless. After removal of the butyl alcohol layer into another tube, it is chilled by immersion in ice-water for a few minutes, and the cloud of water which forms is removed by centrifuging for 1 min.. If this last process is not carried out, the solutions are liable to cloud on cooling.

Under working conditions, batches of eight tubes of plasma are put through the process together; the manipulations after digestion occupy about one hour.

Notes.

1. When solutions of Evans blue in differing concentrations of hydrochloric acid are shaken with equal volumes of butyl alcohol, 99% of the dye passes into the alcoholic phase if the initial strength of the acid exceeds 0.4 N, but remains in the aqueous phase if the strength is less than 0.0001 N. Solutions of dyo in plasma after digestion appear to behave in accordance with pH in the same way, no dyo passing into butyl alcohol if the pH is above 3.

2. It is desirable to wash at a low pH firstly because the removal of plasma pigments is more efficient, and secondly because the precipitate formed is liable to be bulky if the pH is greater than 5 or less than 3. The 0.35 c.c. of 5 N NaOH recommended gives a pH of 3.5 in normal plasma.

3. With this technique of washing, the optical density of the final extract from undyed plasma is about 5% of that from plasma dyed to a degree usual in blood volume estimations.

4. It is possible that in pathological conditions, for example in hypoproteinaemia, the quantities of acid and alkali added may require alteration to give the correct pH at the different stages.

5. Butyl alcohol and aqueous solutions are mutually soluble. The degree of this solubility is affected considerably by the HCl content of the aqueous phase, slightly by its salt content and by temperature, and not detectably by pepsin or by Evans blue. The accuracy of estimation of the dye depends on the uniformity of volume of the final extracts. Therefore all tubes of one experiment must be treated identically with reagents, digestion and washings; thus, if the technique given below for haemolysed samples is necessary for any sample, it should be used for all tubes. The use of wet butyl alcohol for the final extraction has the advantage that its volume only increases by about 4%.

Hæmolysis. The extraction and estimation of the dyo by the method described above cannot be carried out if there is more than a very slight degree of hæmolysis.

Any free hæmoglobin which is present in the sample of plasma is converted into acid hæmatin during the process of digestion; small traces of such hæmatin are removed satisfactorily by the ordinary process of washing, but larger amounts become adsorbed on the precipitate at the interface. If this happens, washing at *pH* 3.5, even if repeated many times, does not remove the adsorbed pigment completely; it is, however, eluted by the strong acid employed at the last stage and thus contaminates the final extract of dye.

Hæmatin itself, being a weak acid and readily soluble in butyl alcohol should easily be removable together with the normal plasma pigments by washing with butyl alcohol at low *pH* if it were not for the presence of the adsorbing precipitate; the separation of hæmatin from dye in the absence of precipitate can be achieved by the following procedure.

5 c.c. of plasma are digested as usual; after addition of 0.2 c.c. of 5 N sodium hydroxide they are washed three times with 5 c.c. of a mixture of equal parts of butyl alcohol and ether*; the washings are discarded and the aqueous layer treated with 3.5 c.c. 6 N hydrochloric acid and 5.5 c.c. butyl alcohol-ether; the mixture is thoroughly shaken and centrifuged. The upper layer, containing dye still mixed with hæmatin, is removed and 5 c.c. are measured into a centrifuge tube together with 5 c.c. of a solution prepared by mixing 86 c.c. *M*/5 potassium hydrogen phthalate with 14 c.c. 5 N sodium hydroxide; the mixture is shaken and centrifuged, when the dye passes into the aqueous layer (which attains a final *pH* of about 4) while most of the hæmatin remains in the butyl alcohol-ether; the latter is discarded and the aqueous solution freed from the last traces of hæmatin by washing once more with 5 c.c. of the alcohol-ether mixture. Finally the aqueous layer is treated with 3.5 c.c. of 6 N hydrochloric acid and the dye is transferred to 5 c.c. of wet butyl alcohol as in the last stage of the ordinary process.

Not only is hæmatin efficiently removed by this process, but also plasma pigments are more completely washed out than by the method given on page 312 so that the final solutions from undyed plasma with or without hæmolysis have nearly the same optical density as butyl alcohol. It is clear therefore that the procedure represents the technique of choice if the final estimation of the dye is to be made with a simple colorimeter; it may indeed ultimately prove to be the best extraction method however the dye is estimated. The process is however slightly more complicated than the ordinary method and moreover the uncertainty regarding the fraction of the total extract represented by the aliquot makes it impossible to calculate the dye content of the blood from the absolute density of the final extract; this means that a reference sample of plasma containing a known amount of dye must always be carried through the process in parallel with the

* The use of the butyl alcohol-ether mixture makes it possible to wash at a lower *pH* and thus to remove a larger proportion of hæmatin. Moreover, the subsequent transference of the dye to the phthalate buffer, which is incomplete from solution in butyl alcohol, is made quantitative by dilution of the latter with ether.

experimental samples. For these reasons therefore we suggest for the present that the modified procedure be adopted only when it is necessitated by the presence of hæmolysis.

The modified method has been tested with plasma samples to which lysed red cells have been added. These samples have been prepared by adding 0.1 to 0.5 c.c. of a 50% aqueous solution of red cells to 5 c.c. portions of plasma, and thus correspond to lysis of approximately 1 to 5% of the red cells from which the plasma was separated. With these increasing degrees of hæmolysis there is a slight increase in the residual hæmatin and a more serious deficiency in the recovery of the dye; the loss of dye amounts to approximately 9% per 1% hæmolysis, whilst the error due to unextracted hæmatin is not more than one tenth of this and is therefore insignificant. The incomplete recovery of dye will lead to an overestimate of the blood volume, but as so gross a degree of hæmolysis as 1% should never occur clinically the error from this source will not be serious.

Standards and controls.

For the accurate determination of plasma volume, not only must extracts be prepared from the samples of plasma drawn after injection of dye, but also "control" and "standard" samples of plasma should be digested and extracted in exactly the same way. The "control" is 5 c.c. of plasma taken before injection of the dye. The "standard" is another 5 c.c. of this plasma to which is added 0.5 c.c. of a 1:50 dilution of the stock dye. Now the standard dose of stock dye used in these experiments was 5 c.c.. If an extract prepared from the standard exactly matched a plasma sample, each contained the same amount of dye. That is to say, 5 c.c. of plasma contained $\frac{1}{50} \times 0.5$ c.c. of stock dye or $\frac{1}{500}$ th of the amount given; the whole of the dye would in this case be contained in 2,500 c.c.—giving the apparent plasma volume.* Any colour due to incomplete removal of natural pigment or to residual dye from a previous injection is measured in the "control" extract and taken into account, as will be shown later.

The measurements of colour density of the control and standard samples in different experiments do not vary widely if a constant technique is used. This means that, with some sacrifice of accuracy, a blood volume determination could be made by measuring the dye content of a single sample taken 10 min. after injection. The error that would thus be added would be 7% (standard deviation).

Estimation of the dye.

Three instruments have been tested for estimating the dye in butyl alcohol solution:—(1) A simple photoelectric device. (2) Pulfrich step photometer. (3) Simple colorimeter. All the results given here were obtained with the photoelectric device, but most were checked with the photometer, which gave concordant results.

* A weaker standard containing 0.4 c.c. of dye, and corresponding to an apparent plasma volume of 3,125 c.c. was used in blood volume determinations on large subjects.

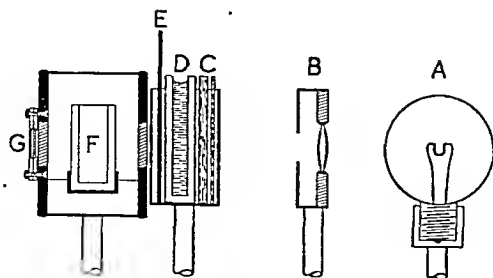
(1) *Simple photoelectric device.**

Fig. 1.

- A. Source of illumination :—12 V., 24 W., silvered lamp with small coiled U-filament, run off six 2 V cells with high acid content to give constant discharge.
- B. Optical system :—condenser giving parallel beam ; iris diaphragm.
- C. Filters :—Wratten 25 red gelatine filter, combined with 2 mm. thickness of "Calorex" glass infrared absorber, manufactured by Messrs. Chance.
- D. $\frac{1}{2}$ cm. glass trough containing water to absorb heat.
- E. Light interrupter :—black card to give zero reading with no light falling on the cell.
- F. Glass trough to hold test solution, 10 mm. depth, optically ground.
- G. Photocell :—selenium barrier cell, 1 cm. square. Wender (10).
- F. and G. mounted in a dark box with hinged lid, so that the apparatus can be used in a lighted room.

Needle galvanometer :—150 divisions full deflection, sensitivity 2 divisions per microamp. (Pattern U. Cambridge Instrument Co.).

The device is built up as shown in Fig. 1. The filter combination gives maximum light transmission from about 600 to 630 $m\mu$. It is impossible to specify the precise wave length sensitivity more closely as this depends on the quality of light source and photocell sensitivity as well as the filter combination. The arrangement was chosen empirically and gives a density of butyl alcohol solutions of Evans blue corresponding to about 70% of the maximum. Beer's law is closely followed over the range employed both for aqueous and butyl alcohol solutions of the dye. Full scale deflection of the galvanometer is readily obtained with this filter combination. The cell shows slight fatigue phenomenon, but if light is allowed to fall on the cell for about one minute before a reading is obtained, no appreciable error results. The theoretical error of this device is less than $\frac{1}{2}\%$. Readings are taken with the trough removed from the instrument, and are recorded as "incident light." The trough is then placed in position containing the solution to be tested, and the galvanometer reading again recorded. The incident light reading is again taken to ensure that no change in light intensity has occurred, and finally a zero reading is made with no light falling on the

* We have to thank Dr. S. S. Yudkin of University College Hospital for advice, and Mr. A. J. Honour for the construction of this instrument.

cell. The density of the trough and dye solution is given by \log_{10} (incident light reading \div reading with trough in position). Since densities are additive, the density of dye solution alone is obtained by subtracting from this figure the density of trough + solution in which the dye is made up, which in the blood volume estimation is the "control" extract. The densities and calculation of results in a typical experiment are set out below.

TABLE 1.

Subject No. 13. Sex F. Age 21. Weight 65.1 kg. Height 178 cm.
Dose 12 mg. Evans blue.

Sample Min. after dye.	Hæmatocrit.	Photocell readings			Pulfrich photometer readings		
		Optical* density.	Plasma vol. litres.	Blood vol. litres.	Optical* density.	Plasma vol. litres.	Blood vol. litres.
Standard†	—	0.207	[3.125]	—	0.27	[3.125]	—
12	36.9	0.216	3.00	4.76	0.29	2.9	4.6
22	36.6	0.228	2.84	4.45	0.29	2.9	4.6
65	37.8	0.221	2.93	4.71	0.28	3.0	4.8
Average			2.92	4.64		2.9	4.7

*The figures represent observed densities less than that of the control extract.

†0.4 c.c. of 0.048 mg./c.c. solution to 5 c.c. plasma.

(2) *Pulfrich photometer.* The instrument makes use of colour filters which used by the eye give fairly narrow bands of wavelength sensitivity. For estimation of Evans blue, filter S 61 giving maximum sensitivity at about $610 m\mu$ was used. For blood volume estimations 1 cm. cells were employed, the control extract being placed in one cell and the dye solutions in succession in the other. This instrument gives consistent results having an error of about 2%.

(3) *Simple colorimeter.* Butyl alcoholic extracts of the dye can be estimated with this instrument; good matching is obtained and the tints and intensities are suitable. The method is only valid if the colour of the control is negligible compared with that of the dye extracts. Otherwise error will be introduced unless the dyed sample is identical with a standard. Measured photoelectrically, however, the density of the control colour in our experiments has been less than 10% of that of the dye and where parallel colorimetric and photoelectric readings have been made they have agreed well. It is clear that a colorimeter can readily be used with this technique if highly accurate results are not required.

*Experimental technique.**Apparatus and solutions.*

1. Centrifuge tubes to hold 15 c.c. blood samples. In these tubes is placed 0.6 c.c. of a solution containing 2% potassium oxalate and 3% ammonium oxalate which is then dried off at 60°C. (11).
2. 20 c.c. glass syringes, dried and oiled with paraffin.
3. Venepuncture needles, kept in spirit, and dried by blowing out over a flame with dry syringe.
4. Wintrobe hæmatocrit tubes fitted with rubber caps.
5. Stock solution of Evans blue 2.4 mg. per c.c. made up in fresh 0.85% saline and autoclaved in 50 c.c. rubber capped bottles. Dose 5 c.c. corresponding to 0.2 mg. per kg. in a 60 kg. subject.
6. 5 c.c. record syringe, calibrated to deliver 5 c.c. without rinsing, the same syringe being used throughout. Sterilised by boiling.

Withdrawal of blood and avoidance of hæmolysis. An intradermal bleb of 2% novocaine was used before each venepuncture. The subject gripped the upper arm with the opposite hand, clenched his fist a few times, and venepuncture was then carried out at once. It was thought that the congestion was too brief to affect the hæmatocrits. Hæmolysis was avoided by attention to the following details:—use of the anticoagulant suggested; dry needles and syringes with well fitting joints to avoid bubbles; clean venepuncture; removal of the needle before transferring the blood from syringe to centrifuge tube; prompt mixing by repeated inversion without shaking; and separation of plasma within six hours.

Hæmatocrits. Hæmatocrits were performed on every sample. This is necessary as the value changes with repeated venepuncture. The blood was first carefully mixed by gentle inversion of the tube 20 to 30 times to ensure fair sampling. No precautions were taken to avoid loss of carbon dioxide from the blood.

The Wintrobe tubes were centrifuged at 8,000 r.p.m. for one hour; the radius from spindle to centre of the tube was 7.2 cm., the radial force therefore being 5,200 g.. At this speed packing was incomplete in half an hour, but readings taken at one and 1½ hours were identical. The red cell layer was then clearly translucent. No facilities were available to see whether a higher speed would give more complete packing. Duplicate tubes from a single sample agreed to ½%.

Injection of dye. The syringe was sterilised by boiling and traces of water blown out. Dye was then drawn in and the plunger adjusted to the 5 c.c. mark. On entry into the vein, ½ c.c. of blood was drawn back and then the entire contents of the syringe were delivered over a period of about one minute. The syringe was not rinsed with blood.

In three cases slight staining round the site of injection was subsequently noted. If the full dose of dye is not given into the bloodstream, the apparent blood and plasma volumes will be too high. These three cases are starred in the table of results.

TABLE 2.

Case No.	Sex.	Age.	Wt. (kg.).	Ht. (cm.).	Surfacet area (sq. metres).	Plasma vol. litres.	Blood vol. litres.	Plasma vol.		Blood vol.		Plasma vol.		Blood vol.	
								c.c./kg. weight.	litres/sq. m.	c.c./cm. litre.	litres/sq. m.	c.c./cm. litre.	litres/sq. m.	c.c./cm. litre.	litres/sq. m.
1	M.	30	55.2	168	1.62	2.42	4.18	43.8	75.9	14.4	24.9	1.50	2.59	1.50	2.59
2	M.	20	58.4	174	1.70	2.59	4.24	44.4	72.7	14.9	24.4	1.52	2.51	1.52	2.51
3	M.	21	70.0	170	1.80	3.11	5.40	44.4	77.2	18.3	31.8	1.73	3.00	1.73	3.00
4*	M.	20	73.1	176	1.89	3.15	6.36	43.1	86.9	17.9	36.1	1.67	3.47	1.67	3.47
5	M.	31	81.8	184	2.04	3.73	6.76	46.0	82.6	20.4	36.7	1.84	3.31	1.84	3.31
6	M.	28	61.2	167	1.68	2.67	4.45	43.7	77.8	16.0	26.7	1.59	2.65	1.59	2.65
7	M.	25	70.2	183	1.93	3.23	5.76	46.0	82.0	17.6	31.5	1.67	2.98	1.67	2.98
8*	M.	28	70.5	182	1.90	4.33	7.00	61.4	99.4	23.8	38.5	2.28	3.69	2.28	3.69
9	M.	32	70.6	186	1.93	3.66	6.23	51.9	88.3	19.7	33.6	1.90	3.23	1.90	3.23
10*	M.	30	68.1	168	1.78	2.96	5.09	43.4	74.8	17.6	30.3	1.66	2.86	1.66	2.86
11	M.	20	49.6	168	1.55	2.66	4.62	53.6	93.0	15.9	27.6	1.72	2.98	1.72	2.98
							Average	47.4	82.8	17.9	31.1	1.73	3.03	1.73	3.03
12	F.	22	46.2	152	1.40	2.25	3.56	48.7	77.0	14.7	23.4	1.61	2.54	1.61	2.54
13	F.	21	65.1	178	1.82	2.92	4.64	44.9	71.3	16.4	26.0	1.61	2.55	1.61	2.55
14	F.	23	55.5	158	1.55	2.48	4.13	44.7	74.6	15.8	26.3	1.60	2.67	1.60	2.67
15	F.	32	65.1	176	1.80	2.66	4.31	40.9	66.3	15.1	24.5	1.48	2.40	1.48	2.40
							Average	44.8	72.3	15.5	25.1	1.57	2.54	1.57	2.54

* Some staining round site of dye injection.

† Calculated from Du Bois nomogram (8).

Procedure in normal subjects. The determinations were made on student and medical volunteers, who were all healthy young adults ranging in age from 20 to 32 years. None of the subjects was abnormal in weight or height. No dietary restrictions were imposed, the subjects coming to the laboratory in the morning or afternoon. Estimations were carried out over a period of three weeks during warm weather with room temperature at 20 to 22°C.. The subjects lay on a couch for the first half hour of the experiment, then walked away and returned for the final sample.

First, 30 c.c. of blood were taken to be used for control and standard. 5 c.c. of dye were then given and 15 c.c. blood samples taken at about 10, 20 and 60 minutes after the injection. Times of withdrawal were noted accurately, no sample being taken at less than 10 minutes after injection.

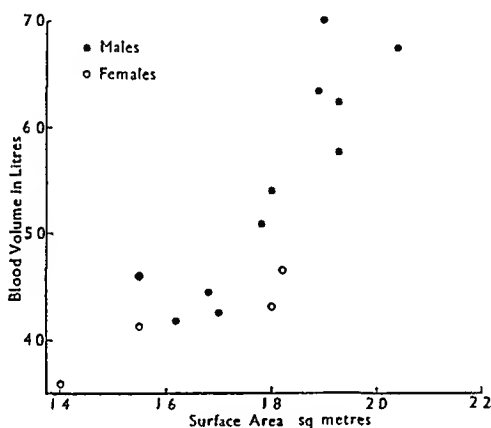


Fig. 2.

TABLE 3.

Comparison of results with those of Gibson and Evans (2).

Plasma vol..			Blood vol..	
c.c./kg..			c.c./kg..	
	Present series.	Gibson and Evans.	Present series.	Gibson and Evans.
Males	47.4	43.1	82.8	77.7
Female	44.8	41.5	72.3	66.1

Results. All these results were obtained by the ordinary method described on page 312, no appreciable hæmolysis having occurred. The cumulative error of sampling, extraction and measurement in this series was $\pm 6.6\%$ (standard deviation). This is too great to allow the disappearance rate of the dye in individual cases to be calculated from the three readings used. Consequently extrapolation to the initial concentration of the dye is impossible and plasma volumes have been calculated by averaging the values obtained from the three samples; blood volumes, calculated from the hæmatocrit of each sample have also been averaged. This procedure would tend to give results some 2% too high if the disappearance rate were 5% per hour. The accompanying table and graph (Table 2 and Fig. 2) summarise the results obtained. The normal value for blood volume cannot be accurately assessed on so small a series, and the results may be compared with the larger series of Gibson and Evans (2) (*see* Table 3). The general validity of the method is evident from these results. When blood volume is plotted against surface area, the scatter from a line in the values for males is much increased by two high figures, Cases 8 and 11. In Case 8 it is possible that the result is faulty owing to leakage of dye under the skin, but this criticism is inapplicable to Case 11. We are inclined to agree with other authors that high plasma volumes in relation to body size are occasionally found in lean muscular individuals.

SUMMARY.

1. A method is described by which Evans blue can be extracted from plasma into butyl alcohol.
2. The dye can readily be estimated photoelectrically or colorimetrically in the final extract, which is free from plasma pigments and opacity.
3. The technique has been applied to the estimation of blood volume in fifteen normal individuals.

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THYROTROPIC HORMONE IN THE BLOOD.

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E. P. SHARPEY-SCHAFER.

Introduction.

THE remarkable "thyrotropic" activity of simple weak acid or alkaline extracts of the anterior lobe of pituitary is well known. The evidence concerning the possibility that anterior pituitary function is a factor of importance in the pathogenesis of Graves' disease is reviewed by Cope (3). He concludes that, while the pituitary theory of origin "is a tantalizingly attractive one, yet conclusive evidence supporting such a hypothesis has remained persistently lacking." One objection to this theory, is that the thyrotropic hormone content of the blood has not been shown to be increased in thyrotoxicosis (6, 7, 3). The adequacy of current methods of estimating the thyrotropic hormone in the blood is, however, far from clear and this aspect of the problem is examined in the present paper. The results of a further series of assays in Graves' disease and allied conditions are also presented.

Experimental data.

Since chemical methods of measuring the thyrotropic hormone content of the blood are not available, the generally less accurate technique of biological assay must be employed. In the present investigation, Fellinger's method (6) was used to separate thyrotropic substance from the blood. The resulting extract was injected into the day-old chick. Activation of the thyroids was the criterion of thyrotropic potency in the extract.

Fellinger's method of blood extraction removes protein material and iodine, but does not seriously affect thyrotropic potency. In each of our assays for thyrotropic hormone 60 c.c. of venous blood was used. Equal portions of the resulting extract were injected into four chicks, in five divided doses on consecutive days. Each chick thus received the equivalent of 15 c.c. of blood. The chicks were killed on the 6th day. Immediately

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afterwards the thyroids were removed, weighed and fixed. A standardised technique of preparing and injecting the extracts and of preparing and staining the histological sections was used throughout.

Iodine when given simultaneously with thyrotropic extract tends to promote colloid storage in the thyroid follicles and may thus mask the activating effect of the extract (5, 11, 17). Hence the elimination of iodine is important in assays for thyrotropic hormone. This requirement is not fulfilled when whole blood or serum is injected, for their iodine concentration may be much increased particularly in patients under iodine therapy. Fellingner claimed that with his method no iodine passes over into the final aqueous extract, and we have been able to verify this by direct estimations of the iodine in seven of our extracts chosen at random. Each showed a negligible iodine content. Three of the patients concerned were taking Lugol's iodine.

The thyroid glands of chicks injected with blood extracts were compared with those from 54 uninjected chicks killed on the 6th day, and with the glands from groups of chicks injected with varying dilutions of a test preparation of thyrotropic hormone. In normal chicks the histological appearance of the thyroid is very constant. The glandular structure is strikingly simple and uniform throughout, being composed of well filled follicles uniformly distended with evenly staining colloid and lined by flattened epithelium. There is a minimum of stromal and lymphoid elements and no central "hyperactive" area such as frequently occurs in the normal guinea-pig. The latter appearance may lead to erroneous conclusions in assay work unless great care is exercised, but the same difficulty does not arise with the day-old chick.

The injection of different dilutions of a thyrotropic pituitary extract (Ambinon*) afforded an opportunity of studying the different degrees of histological activation in the chick. At the beginning of the experiments all the ampoules of extract were pooled to ensure constancy in the strength of the test preparation. Various dilutions were then made as shown in Table I. Groups of chicks, six in each group, were injected with the different dilutions. The same method of dividing the total dose of extract and of preparing histological material was used as in the case of chicks injected with blood extracts.

The earliest sign of thyroid activation is hypertrophy of the follicle cells (9). A measurable increase in the height of the cells can readily be detected by the practiced eye, and more reliance can be placed on this change than upon other criteria of activation commonly used (12). Thyrotropic potency was detectable in dilutions down to 1:6000. In these and all subsequent experiments thyroid activation in two chicks of any group was considered adequate evidence of potency in the injected solution. If, in any chick, thyroid activation was doubtful, the result was called negative.

* Kindly supplied by Organon laboratories.

TABLE I.

Assay of test preparation of thyrotropic extract.

Solution number.	Dilution.	Average thyroid weight in 6 injected chicks.	Histological* stimulation.
1	1 in 36	18.15 mgms	+++
2	1 in 50	14.8 mgms	+++
3	1 in 75	10.4 mgms	+++
4	1 in 100	9.3 mgms	+++
5	1 in 125	9.6 mgms	+++
6	1 in 250	5.8 mgms	++
7	1 in 500	5.8 mgms	++
8	1 in 1,000	4.8 mgms	+
9	1 in 1,500	4.4 mgms	+
10	1 in 3,000	4.0 mgms	+
11	1 in 6,000	3.4 mgms	+
12	1 in 8,000	3.2 mgms	—
13	1 in 12,000	2.9 mgms	—

* We have found useful a rough histological grading (+++, ++ and +) to indicate different degrees of activation in the chick's thyroid. Thus +++ stimulation indicates an extreme degree of cellular hypertrophy and hyperplasia, excessive vascularity and complete resorption of colloid; in grade ++, the changes, though still striking, are less marked. The cell height is less, the cytoplasm stains more deeply and some colloid is seen in the follicles. In grade +, the evidence of stimulation though definite is not so obvious. The cells must be deliberately examined when they will be found to be hypertrophied. There are signs of colloid resorption and increased vascularity.

The table also shows the more or less linear increase in the weight of the chick's thyroid, resulting from the injection of increasing concentrations of thyrotropic hormone, previously noted by Smelser (14).

Experiments on the efficiency of the extraction method may now be considered. To a specimen of normal blood, diluted test solution was added to make a final concentration of 1:5000 thyrotropic extract. The blood was thoroughly shaken and then extracted, but the extract failed to cause activation of the thyroids in any of the four chicks injected. Reference to Table I shows that some of the potency of the mixture must have been lost in the process of extraction. The experiment was then repeated except that thyrotropic extract was added to make a final concentration of 1:3000. The resulting blood extract was potent in 3 out of 4 chicks. It may be concluded that some of the hormone is lost in extraction by Fellingner's method, but this disadvantage is offset by the fact that the equivalent of a

larger quantity of blood is injected than would be possible if serum or whole blood were used.

The following groups of subjects were tested.*

Normal controls. Blood assays for thyrotropic hormone were negative in all 12 normal subjects tested. Four of the controls were men, and the remaining eight, women. Three of the latter were menopausal.

Normal and myxœdematous subjects injected with thyrotropic extract. Undiluted thyrotropic extract from the same pool as was assayed on chicks was injected into two normal and two myxœdematous subjects. The effect of thyrotropic hormone on the normal human subject has been fully described by Sharpey-Schafer and Schrire (13). Normal controls injected with 1 c.c. of a similar thyrotropic preparation daily, for 3-6 days, responded by developing enlargement of the thyroid gland, tachycardia, raised basal metabolic rate and creatinuria. In patients with myxœdema there was no response.

Our first normal subject was injected with 4 c.c. of undiluted test preparation. Within 36 hours he developed a palpable goitre and increase in pulse rate of 40 beats per minute. Blood samples were taken before the injection and at 1, 4½, 11 and 24 hours afterwards. The second normal subject, injected with 2 c.c. of extract daily for 3 days, gave a similar clinical response, maximal after 86 hours. Blood samples were taken before the first injection and afterwards at 2, 24 and 86 hours. Assays of all the blood samples taken in these two subjects were negative. One of the myxœdematous patients was injected with 4 c.c. of the test preparation in a single dose, the other with 2 c.c.. In the first, blood samples were taken 20 and 72 hours after the injection, in the second, 20, 72, 120, 168 and 216 hours afterwards. Neither patient showed any clinical response to thyrotropic extract and here, also, all the blood assays were negative.

Simple goitre. In three patients with simple goitre blood assays were negative.

Thyrotoxicosis. Tests were done on 11 patients with thyrotoxicosis. In all cases, hyperthyroidism was of moderate intensity or severe. Exophthalmos or lid retraction was present in 7 of the 11 patients. In two cases, the assays were positive and in the remaining nine, negative. In one of the two patients with positive assays, thyrotoxicosis was of exceptional severity; in the other, there was extreme proptosis (exophthalmometer readings (Hertel), right eye, 26 mm.; left eye, 28.5 mm.) and lid retraction, but the actual thyroid toxæmia was mild. In the latter case the assay was repeated with a confirmatory positive result.

A small group of four patients with recurrent thyrotoxicosis was also tested. In each case there was a palpable regrowth of thyroid tissue and

* The authors thank the staff of the Westminster Hospital, the London County Council thyroid Clinic and the British Postgraduate Medical School for permission to investigate cases in their charge and to publish these investigations.

unequivocal evidence of hyperthyroidism. In one case, the assay was strongly positive ($++$), in a second positive ($+$) and in the remaining two cases, negative. In the first case ($++$) there was an unusually large regrowth and severe thyrotoxicosis but in the second case ($+$) there were no noteworthy features.

Hypothyroidism and myxædema. Three patients with spontaneous myxædema and five with post-operative hypothyroidism or myxædema were tested. In no case was thyroid extract being given. One of the patients with spontaneous myxædema and two of those with post-operative myxædema gave positive results. In one of the latter the result was $++$. In three patients, in whom post-operative thyroid deficiency was incomplete, the result was negative (confirmed in one case).

In two patients in whom the assays were at first positive ($++$ and $+$) the results became negative after thyroid therapy. The change agrees with the concept of pituitary-thyroid interaction suggested by the experimental findings of Marine, Rosen and Spark (10). Our data, however, are very limited and it must also be admitted that nothing is known regarding the constancy with which positive results can be expected if the test is repeated on such patients untreated with thyroid extract.

Severe proptosis and ophthalmoplegia. Four patients were included in this group. In one, unilateral proptosis, partial ophthalmoplegia and lid retraction of the Graves' disease type were present but no other signs of thyrotoxicosis. The assay was negative. The second patient had developed severe proptosis, palpebral and conjunctival œdema and epiphora, for the first time, some 3-4 months after thyroidectomy. On two occasions, the test gave negative results. In the third patient, proptosis had increased markedly after thyroidectomy (pre-operative exophthalmometer readings (Hertel); R., 19, L., 20; 9 months afterwards; R., 25.5, L., 26.5) and ophthalmoplegia had developed. There was a mild degree of post-operative hypothyroidism. The blood assay was positive ($++$). In the fourth patient severe proptosis, chemosis and ophthalmoplegia were present prior to thyroidectomy. Operation relieved the thyroid toxæmia, but the eye signs were unchanged. Assays were done two and eight months after operation. In both, the results were positive.

Discussion.

The combined use of the day-old chick as test-object and Fellinger's method of blood extraction appears to provide the most practical and sensitive method of assay for thyrotropic hormone in the blood at present available. There is of course most powerful evidence, reviewed by Cope (3), that thyrotropic hormone is present in the blood in health. The low concentration in which it circulates is, however, beyond detection by present methods of measurement. Thus in twelve normal subjects, our blood assays were negative. Similarly, it has been shown that while it is possible

to produce thyrotoxicosis in man experimentally by injecting a thyrotropic substance, the latter cannot be detected in the blood. A 1 : 6000 solution of our thyrotropic pituitary extract, or an extract of normal blood to which the extract had been added to make a concentration of 1 : 3000, was capable of activating the day-old chick's thyroids. Since as little as 2 c.c. daily of the test preparation was sufficient to produce experimental thyrotoxicosis in man, it is not surprising that its concentration in the blood was insufficiently raised to give positive results. It is also clear that inability to detect circulating thyrotropic substance in cases of thyrotoxicosis would not necessarily mean that it plays no part in the pathogenesis. It has been suggested as a possible explanation of the negative results of assays, that thyrotropic substance is fixed or in some way altered by the thyroid gland that is being stimulated. When, however, doses of thyrotropic extract sufficient to cause stimulation in normals were given to subjects with myxœdema who had no thyroid tissue it was still impossible to recover the extract from the blood.

Certain data relating to the different assays may conveniently be considered at this point.

TABLE II.

Technique of assays of blood thyrotropic hormone in thyrotoxicosis.

Author.	Date.	Test-object.	Material injected.
Fellinger	1936	Guinea-pig	Extract = 5—8 c.c. blood.
Hertz and Oastler	1936	Hypophysectomised rat	Serum
Cope	1938	Guinea-pig	Serum
Collard, Mills, Rundle and Sharpey-Schafer.	1940	Day-old chick	Extract = 15 c.c. blood.

Though previously widely used for assaying thyrotropic pituitary extracts, the guinea-pig is now known to be insensitive compared with the day-old chick (14, 4, 1). Moreover, with the guinea-pig the diagnosis of thyroid activation is open to fallacy because of the central "hyperactive" area which may occur normally. The hypophysectomised rat is, however, an important test-object and ideally the hypophysectomised animal should be used in all assays for pituitary hormones, in order to avoid secondary effects produced by the injected material in the function of the animal's own pituitary. The technique of hypophysectomy is, however, difficult and renders the use of hypophysectomised animals in extensive assay work impracticable. Moreover, Jones (8) found the hypophysectomised rat less sensitive than the day-old chick to injected thyrotropic extracts. Thus it

seems clear that the technique used by us is at least as sensitive as any of those previously employed. We may, therefore, conclude that failure to detect thyrotropic substance in previous assays does not exclude the anterior pituitary as a factor of importance in Graves' disease. In the past negative results have certainly been credited with too much significance.

In spite of its deficient sensitivity, the present method occasionally gave positive results in patients with thyrotoxicosis (4 out of 15 cases). Included in this number were four cases of recurrent thyrotoxicosis in two of which positive results were obtained. The number of cases is clearly too small to attempt any correlation between the severity of the thyroid toxæmia or eye signs and the occurrence of a positive assay. The menopause was not a factor in the positive cases. The results suggest, however, that, contrary to previous reports, the thyrotropic hormone content of the blood may be increased in Graves' disease, but too far-reaching conclusions should not be drawn from the data at present available.

Previous workers have obtained positive results from blood assays for thyrotropic substance in myxœdema (6, 7, 3), and as shown in one of our cases it may be present in considerable concentration. Hertz and Oastler (7) appear to have obtained remarkably uniform results, all nine of the patients tested giving positive assays. In fact, their positive results in myxœdema and negative results in thyrotoxicosis accorded with the generally contrasting features of the two conditions. It need hardly be stated, however, that there is no *a priori* consideration suggesting that the functional activity of the anterior pituitary should be opposite in direction in the two conditions. In particular the undoubtedly positive findings in post-operative myxœdema should not be used to support the thesis that anterior pituitary function is depressed below normal prior to thyroidectomy.

The unsatisfactory nature of the evidence gained from blood assays is nowhere better illustrated than in the group of patients with severe proptosis and ophthalmoplegia. There is strong evidence, reviewed by Brain (2), suggesting that the anterior pituitary is of importance in the pathogenesis of such conditions, and this view has been made even more attractive by more recent experimental data (15, 16). Yet in one patient two assays, done during a period when proptosis was measurable increasing, both gave negative results. The third case in this group was very similar clinically yet the result was strongly positive. It seems wisest to conclude that the conflicting results of the blood assays are due to imperfection of our method of assay rather than to any essential difference in the behaviour of the anterior pituitary in the two cases.

SUMMARY.

1. The thyrotropic hormone content of the blood was assayed in a variety of clinical conditions.

2. In twelve normal subjects the assays were negative. A test preparation of thyrotropic hormone was injected into normal subjects producing a state of experimental thyrotoxicosis. Blood assays at different stages of the clinical response were negative.

3. In four out of fifteen cases of thyrotoxicosis positive results were obtained. Positive results were also found in spontaneous and post-operative myxoedema and in cases of severe proptosis and ophthalmoplegia after thyroidectomy. Positive findings were, however, inconsistent and no far-reaching conclusions can be drawn regarding the importance of the anterior pituitary in the pathogenesis of these conditions.

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AN INSTRUMENT FOR MEASURING THE QUANTITY OF BLOOD AND ITS DEGREE OF OXYGENATION IN THE WEB OF THE HAND.

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School.*)

Method.

Principle. If a beam of light shines through a fold of skin, the proportion of the light transmitted depends on the amount absorbed by the skin itself and on that absorbed by the contained blood. If the amount absorbed by the skin alone can be measured directly, the light absorbed by the blood can be found. In infra-red light, the absorptions of infra-red light by reduced and oxygenated blood does not differ appreciably; the quantity of blood present can therefore be measured. In red light, however, reduced absorbs more light than oxygenated blood; this property is used to measure the degree of oxygenation.

A photoelectric instrument is now to be described, which measures the light transmitted through the web of the hand alternatively in either the red or infra-red spectral regions. The instrument has been calibrated with films of whole blood, so that the web measurements can be interpreted to give values for oxygenation in terms of percentage saturation, and for quantity of blood in terms of thickness of whole blood.

Apparatus. Caesium emission gas filled type of photocell, sensitive to red and infra-red light (Cetron C.E.1).

Gelatin colour filters, red and infra-red (Wratten No. 29 and double thickness of No. 87).

Skin clamp embodying light source (4 volt torch bulb) and capsule for compressing the web.

Pressure reservoir and mercury manometer for inflating pressure capsule.

Simple battery valve amplifier built up to contain H T and L T batteries, the latter also supplying current for the light source.

* Aided by a grant from the Shock Committee of the Medical Research Council.

Needle galvanometer (Cambridge Instrument Co., pattern U), provided with various sensitivity scales for current and voltage measurement. The photocell and skin clamp are assembled as in Fig. 1. The colour filters are held in a slide (B), by which one or the other is moved across into position. Another slide (A) either allows the transmitted beam to fall upon the photocell, or prevents any light from reaching it.

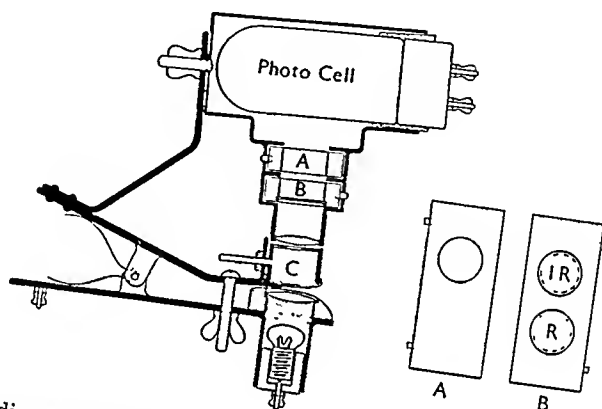


Fig. 1. Sectional diagram of photocell and skin clamp.
 A = slide holding colour filters, red (R) and infra-red (I.R.).
 B = slide for transmitting or cutting off light to photocell.
 C = pressure capsule.
 On the right the slides A and C are shown viewed from above.

The skin capsule, inflation of which renders the skin bloodless, is essentially that of Grant and Rothschild (1), but a more durable membrane is used. This consists of an inner layer of thin transparent latex rubber, and an outer layer of Cargile goldbeater's skin, punctured in one place, and softened with glycerine. The rubber remains airtight, while the Cargile prevents over-distension.

The amplifier (Fig. 2) serves to convert into an easily measured current the small changes resulting from illuminating the photocell. The circuit is complicated at two points only. Adjustment of the grid bias rheostat R 2 enables the best part of the valve characteristic to be selected as the working range. In this way, and by choice of a suitable valve (Mazda Pen 220), an approximately linear relation is obtained between light intensity and changes in galvanometer readings. The rheostat R 2 (compensator) is then adjusted so that the galvanometer reads zero when no light is falling on the photocell. The use of batteries to supply the amplifier is advantageous in that the current supply from the mains may be liable to fluctuate.

Method of use. The clamp is fixed on the web of the hand between the thumb and forefinger. The lamp is switched on, and the current flowing

through it measured on the galvanometer and adjusted to a standard value with a rheostat. The galvanometer is switched over to the amplifier, and valve and compensation current switched on and adjusted as already described. These operations occupy about two minutes. The slide is now moved over to allow the light transmitted through the web to reach the cell, and readings are taken in red and infra-red light. The skin capsule is inflated to a pressure of 200 mm. of mercury, thus expelling blood from the skin flap under examination. Readings are again taken with both colour filters. A zero reading is taken as a routine between each pair of measurements, as some drift occurs even with good batteries. During a prolonged experiment, the lamp current is also checked from time to time, and adjusted if necessary.

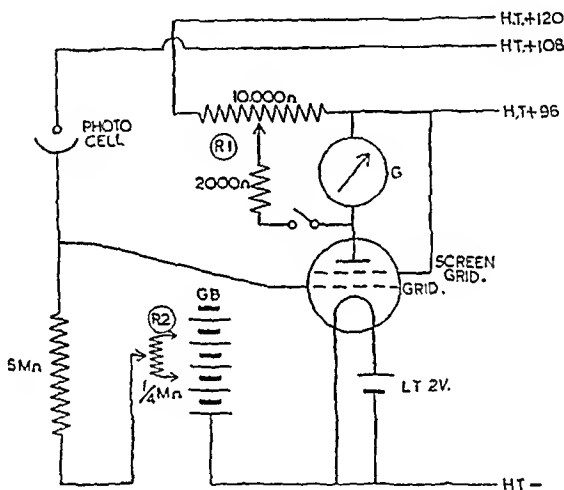


Fig. 2. Amplifier circuit. R_1 = compensator. R_2 = fine adjustment of grid bias.

Calculation of results. The galvanometer readings give measurements of the light transmitted through the web or blood film. Now optical density $= \log \left(\frac{1}{\text{transmission}} \right)$. Optical densities are additive in property; the density of blood is obtained from the measurements of light transmission by the web, and by the web rendered bloodless, by taking the difference between the two densities—that is to say, the difference between the logarithms of the readings. This is the same as the logarithms of the ratio of the readings, a figure conveniently obtained with a slide rule.

Calibration. Blood films of various thicknesses were prepared in special slides. These were made by attaching one to six layers of coverslips to each end of an optically ground glass slide and laying another slide on the top. The thickness of each film was measured accurately with a dial micrometer

10 c.c. of blood was drawn and mixed under oil with dried oxalate (0.8 c.c. of 2% potassium oxalate and 3% ammonium oxalate). Oxygenated and reduced blood samples were prepared by passing oxygen or pure carbon dioxide over specimens in a tonometer, the gas having been previously moistened by bubbling through 0.85% saline. The films were made up quickly without appreciable exposure to the air and then waxed. This prevents evaporation (which would crenate the red cells) and also reoxygenation in the case of the reduced blood.

Measurement of the optical density of thin blood films was carried out in red and infra-red light as in the case of the web. A slip of paper was placed immediately beneath the blood films to act as a light diffuser; for in the web, the skin similarly diffuses light. Finally, a measurement of the density of the paper and glass slides containing no blood was made to enable the density of the blood alone to be found. This is exactly analogous to the measurement of the density of the skin rendered bloodless by compressing with the capsule.

TABLE I.
Calibration.
Normal blood Hb 112%.

BLOOD.	Film thickness (mm.).	Readings.		Blood density = $\log \frac{\text{control reading}}{\text{film reading}}$		Ratio of densities. Red/Infra-red	Density of blood 1 mm. thickness.	
		Red.	Infra-red	Red.	Infra-red		Red.	Infra-red
Oxygenated	0 (control)	94.8	93.8	0	0	—	—	—
	.397	52.7	48.8	.255	.284	.898	.643	.715
	.464	46.7	43.0	.308	.339	.904	.664	.730
	.667	34.8	29.6	.435	.501	.866	.652	.751
	.768	32.0	25.9	.472	.559	.842	.670	.728
						Av. .887	.660	.731
Reduced*	0 (control)	98.2	97.9	0	0	—	—	—
	.310	49.4	56.3	.298	.240	1.242	.961	.774
	.430	38.0	45.3	.412	.335	1.230	.958	.779
	.693	24.0	30.8	.612	.502	1.218	.883	.723
	.784	21.2	27.9	.666	.545	1.221	.849	.718
						Av. 1.228	.913	.749

* 4% oxygenated (Van Slyke).

Results.

Results of calibration experiment. If the densities of blood films are plotted against their thicknesses, the graph is not precisely linear, whether the measurements are made in red or infra-red light. But the divergence from linearity is similar in each case, and the ratio of the densities is constant over the physiological range of blood thickness found in the web. The size of this ratio depends on the degree of oxygenation of the blood. Thus the ratio of density in red light to that in infra-red for fully oxygenated blood is 0.89, while for reduced blood, this ratio is 1.23. Assuming that intermediate degrees of oxygenation behave as mixtures of oxygenated and reduced blood, the degree of oxygenation of any sample can be calculated.

The figures obtained in a calibration experiment are given in Table I, and plotted in Fig. 3.

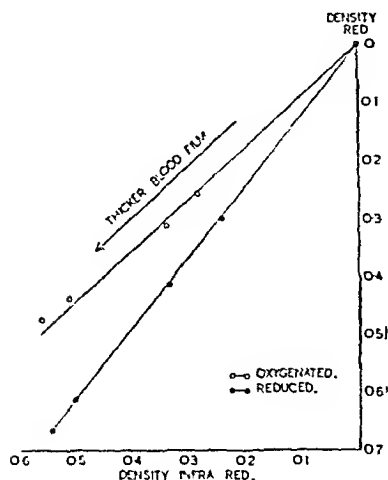


Fig. 3.

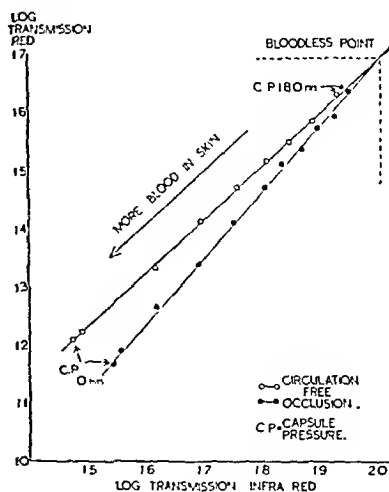


Fig. 4.

Fig. 3. *In vitro* calibration. The densities of various thicknesses of blood film in red light is plotted against their densities in infra-red. Reduced and oxygenated blood films.

Fig. 4. *In vivo* experiment. The amount of blood in the web of the hand is diminished in steps by inflation of the capsule to pressures up to 180 mm. of mercury. The logarithm of the red light transmission at each stage is plotted against the logarithm of the infra-red transmission. Partial reduction of the blood in skin obtained by occluding the circulation with a pressure armlet for 15 min., and keeping the hand warm.

Results of measurements on the web. Although there is no proof that blood in the vessels of the skin has the same optical properties as in the films between glass slides, calculations based upon this assumption give reasonable results. In Fig. 4, transmission readings from the web are plotted logarithmically so as to correspond to the calibration densities in Fig. 3. In this experiment, the quantity of blood in the skin was diminished by inflating the capsule in steps. Reduction of the blood was produced by

occluding the circulation to the arm with a pressure cuff, and keeping the hand warm for fifteen minutes. The readings with various capsule pressures were then repeated. This experiment also provides a test of satisfactory removal of blood from skin by the capsule. Since the density of the blood in red light is different when reduced or oxygenated, the point when the lines intersect in the figure must correspond with the bloodless state of the skin. With the capsule inflated to 180 mm. Hg, 90% of the blood has been expelled.

The density ratio that gives the degree of oxygenation can also be measured from the gradient of the graph when logarithmic plotting of the light transmission is used. Thus calculations of degree of oxygenation are not vitiated by incomplete blood removal, but quantity calculations will of course be somewhat low.

Further *in vivo* results are given in Fig. 5. This shows the effect of venous congestion, and of arterial occlusion for the different periods of time.

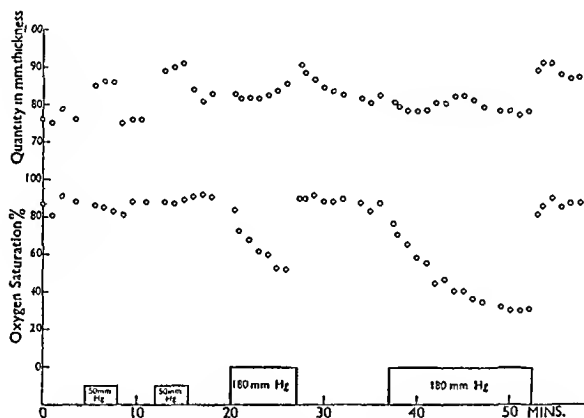


Fig. 5. Results of inflating pressure armlet to 50 mm. of mercury, producing congestion, and to 180 mm. of mercury, occluding the circulation.

Slight reactive hyperæmia increases the blood quantity even after congestion, but is marked after occlusion, and is greater after the more prolonged loss of circulation.

Discussion and previous work.

This apparatus was designed for the study of the peripheral circulation in shocked patients. It was therefore important that it should be portable and easy to use. Certain limitations must be noted. Degree of oxygenation is calculated from successive readings with the red and infra-red filter. If the quantity of blood is changing very rapidly, as during the development of congestion after venous occlusion with a pressure cuff, then the values are incomparable, and therefore the results are invalid.

In the thickness of skin examined also, all kinds of blood vessel are included. The figure for degree of oxygenation is, therefore, a mean value. This may explain why complete reduction of the blood does not occur even after prolonged circulatory occlusion, since some of the blood being in large vessels, may not take part in the respiratory exchange. Further, the degree of oxygenation of skin blood depends on local factors as well as on the oxygen content of arterial blood. Conditions such as skin temperature must always be taken into account.

Certain advances on previous work have been made and the instrument is made available for clinical use. In vitro calibration with whole blood is certainly desirable, instead of assuming that prolonged occlusion produces complete reduction of the blood (2), which is apparently not the case. Carbon dioxide seems to be the most suitable agent for preparing reduced blood, as chemicals such as hydrosulphite produce other changes in addition.

The colour filters have been chosen empirically, and give good results with the photocell mentioned. Even if blood quantity only is to be studied, a spectral region where changes in oxygenation do not affect the result should be selected by use of a suitable colour filter.

SUMMARY.

An instrument is described by which the light transmission through the web of the hand is measured at two different spectral regions. From this, the quantity of blood present and its degree of oxygenation are calculated. The apparatus is portable and suitable for clinical investigations.*

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* The entire instrument was constructed for me by Mr. A. J. Honour, whom I wish to thank.

ŒDEMA FOLLOWING ISCHÆMIA IN THE RABBIT'S EAR.

By E. E. POCHIN.

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It is observed that human limbs which have been trapped in debris or otherwise compressed for long periods, may, on release, develop a massive œdema. The events are frequently complicated by local trauma and vascular damage in the limb or by general disturbances, but the following case, on which measurements were made, illustrates œdema following compression without shock or local wounds.

A woman of 28 was trapped in debris and her right leg was uniformly compressed below the knee for 10 hours. The left leg was at first similarly compressed but was freed after a few hours. An hour after release, the right leg was flushed and somewhat swollen, but the skin was undamaged except for slight grazing. The left leg appeared normal. The right leg continued to swell for 24 hours, when it exceeded the left in volume by 270 c.c. (13%) between knee and ankle, and the œdema pitted on pressure (Fig. 1). The difference between the legs then decreased, and was slight

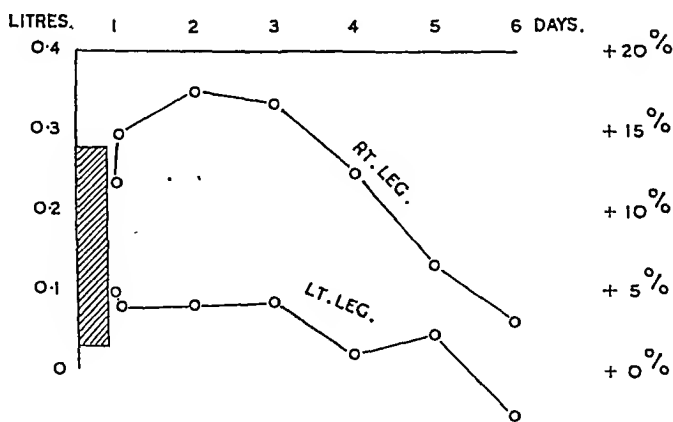


Fig. 1. Volume of human legs between knee and ankle after compression. Period of compression shown by shaded area. Volumes estimated by girth measurements at 2-inch intervals.

* Work undertaken for the Medical Research Council,

after three weeks, although both legs were now reduced in volume by wasting. A subsequent measurement suggested that the left leg had initially been somewhat swollen.

The following investigations were made to determine whether a prolonged arrest of the circulation is a sufficient cause for œdema in the affected parts on release of the circulation. For this purpose the rabbit's ear was used, since the circulation can be arrested at the base without pressure on the distal parts of the ear in which the subsequent changes may be studied. A massive œdema develops, the volume of which can readily be measured, and samples sufficient for protein determination obtained; and yet the total amount of œdema formed is inadequate to cause shock or general disturbance to the animal.

In previous work of this kind a leg has usually been compressed to investigate the general effects of release. It has been found by Allen (1) that gangrene follows if occlusion has continued for about 15 hours in various species, and that a shorter period is effective if the limb is kept warm during the occlusion than if it is cooled. Brooks and Duncan (2) have determined the length of occlusion required to cause gangrene of the rat's tail at various temperatures.

Method.

Occlusion. The ear was depilated by applying for 3 minutes an aqueous paste made from barium sulphide 7, talc 7, flour 7, and powdered soap 1 part. Swelling never resulted, but as a precaution no ear was occluded until several days after depilation. A large wood cork was then placed inside the ear distal to the end of the cartilage, and the skin smoothed over it, not quite covering the whole of the cork. A strip of rubber sheeting, about 1 cm. wide, was wound once round the ear over the cork to which it was secured at each end under moderate tension. The rabbit did not appear to be disturbed by the cork, and when it had been placed in position, returned to its food.

It is important that a wide band should be used, causing moderate and uniform pressure under it. With a narrow band, the pressure is sometimes too low and the circulation is incompletely obstructed; after a few hours, the ear is found to be warm and swollen and the veins are distended. Or the pressure is too high and the tissues of the ear remain indented at the site of the ligature when it is removed; in consequence, the arteries only open gradually across this area, causing distension of the veins distally, until those in the compressed zone also re-open. A wide band produces complete occlusion, and vessels beneath the band open as soon as it is removed.

Measurement of œdema. The formation of œdema after release of the circulation can readily be measured. The thickness of the ear over the central artery is determined before occlusion at various distances from the

tip. Two flat brass discs, each 1 cm. in diameter and 0.5 mm. thick, are placed on either surface of the ear in the required position. The distance between their outer surfaces is found by closing parallel bladed calipers onto them until light contact is made. Such measurements are repeated several times before occlusion, and at intervals after release of the circulation. The ear is about 1.0 mm. thick at 6 cm. from the tip, and about 0.7 mm. thick near the tip. When œdematous, it increases to three or more times its previous thickness, the percentage increase being about equal at different distances from the tip. For the first hour after release the ear is kept horizontal by means of a light harness, but subsequently the weight of œdema maintains this position.

Protein determination. Within an hour of release of the circulation after a long occlusion considerable œdema has accumulated. If the ear is pricked, a large drop of clear colourless fluid forms, and more can readily be expressed. The fluid is sometimes streaked with blood from punctured cutaneous vessels, but this contamination can usually be avoided by rejecting the first drop.

0.05 c.c. of the fluid was taken up in a blood sugar pipette and washed into 2 c.c. of distilled water. The nitrogen content was estimated by Nessler's method (3). The N.P.N. of the fluid was determined on several occasions and found to average 50 mg. per 100 c.c.. All protein concentrations have been based on total nitrogen figures, and are thus high by about 0.3 %.

Results.

On releasing the circulation after a prolonged occlusion, the arteries and veins of the ear immediately fill with blood and become widely dilated. The ear becomes flushed and warm in comparison with the normal opposite ear, the vessels of which remain unaltered. This vascular dilatation in the affected ear may persist for ten days. On one occasion, a branch of the central artery contained cyanosed blood until 30 minutes after release, but the obstruction then ended spontaneously, the artery becoming filled with red blood throughout. In the remaining 63 ears there has been no evidence of thrombosis on naked eye examination.

Oedema begins to develop as soon as the circulation is released, its amount depending on the duration of occlusion. The following are average figures, although the œdema varies substantially in amount in different rabbits.

After 2 hours' occlusion, measurement reveals a slight thickening of the ear to about 120% of its initial value, and this swelling lasts for a few hours and then subsides.

After 6 hours' occlusion, swelling is obvious on inspection and the œdema pits on pressure. The ear is swollen to about 150% of its initial thickness and its vessels are dilated. It returns to normal in a few days.

After 18 hours' occlusion, massive œdema develops, the ear commonly increasing to between 300 % and 600 % of its initial thickness (Fig. 2). The rate of swelling is at first rapid, the thickness of the ear being doubled in 30 minutes; but this rate steadily decreases, so that at 2 hours œdema is present in almost its greatest amount. The ear continues to swell slowly, however, for about 48 hours and then gradually subsides, only returning to normal thickness after several weeks. Dilatation of vessels persists for a week or more from the time of release, but neither vesiculation nor gangrene occur.

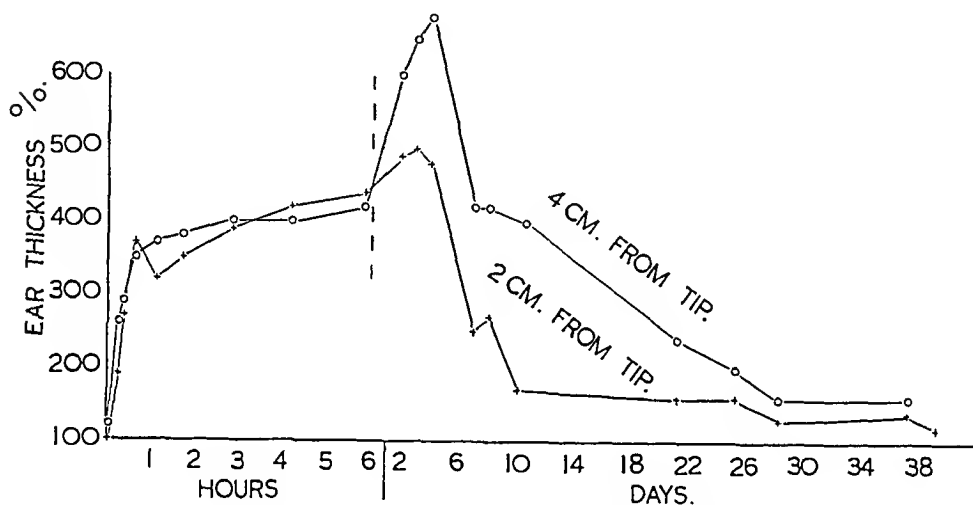


Fig. 2. Thickness of rabbit's ear as percentage of initial thickness, after 18 hr. occlusion at room temperature.

After 24 hours' occlusion, the extent of œdema and the course of events is similar for the first two days, the ear then being grossly œdematous, warm, flushed, and with dilated vessels. Extensive vesicles now form on the skin, and, with loss of their thin superficial layer, exudation follows. By coagulation of this exudate a bulky crust is formed covering the ear, below which the ear itself dries and hardens, and all or part separates after about 3 weeks.

The consequences of circulatory occlusion depend not only on the duration of occlusion but on the temperature of the ear during the period. The figures quoted above refer to occlusion at room temperature. In further experiments the occlusion was performed in a warm room maintained at 88° F.. After occlusion at this higher temperature for 18 hours gangrene is a more frequent sequel, although the œdema is not greater than after equal occlusion at room temperature (*see Table I*).

TABLE I.

Frequency of gangrene in the rabbit's ear after ischæmia.

Temperature during occlusion.	Duration of occlusion.	Gangrene of the ear.		
		None.	Partial.	Complete.
Room temperature	18 hours	8	2	0
" "	24 "	0	3	2
88° F.	18 "	0	1	30

The protein concentration of the œdema fluid is at first high and in seven experiments with occlusion for 18 hours at 88° F. samples obtained one hour after release have contained 5.2% of protein (*see* Table II). The normal plasma protein has been 6.6% by the same method. About 90% of the œdema protein is albumen. The concentration falls during the next 5 hours to an average of 3.5%. Three equivalent figures after occlusion at room temperature have averaged 2.3%.

TABLE II.

Protein concentration (%) in œdema fluid.

Rabbit No.	Hours of Occlusion.	Hours after release.						
		1	2	3	4	5	6	24
<i>Room temperature.</i>								
1	18	—	—	—	—	—	2.3	—
4	18	—	—	—	—	—	2.1	—
5	17	—	—	—	—	—	2.4	—
<i>Warm room</i>								
1	17	5.0	4.1	—	3.6	3.3	—	3.8
2	18	—	—	—	—	—	2.4	—
7	18	—	—	—	—	—	2.7	—
11	18	4.1	—	3.8	—	—	3.9	—
13	18	—	3.9	—	—	—	3.2	2.9
14	17	4.2	4.3	3.2	—	—	4.4	3.2
15	18	4.6	4.1	3.7	3.3	—	3.1	3.1
16	17	5.7	4.9	—	—	4.4	4.1	3.6
23	17	5.3	4.2	3.5	—	3.0	—	3.3
26	16	7.8	7.1	6.3	—	5.0	—	—
9	22	—	4.9	—	—	—	4.4	—

The œdema in the affected ear not only forms distal to the site of the ligature but extends proximally up the ear. The spread is not gravitational, since it occurs if the ear is held with the tip downward, and is presumably due to a raised tissue tension distally. The protein content of this fluid has been found to be lower than that in the area distal to the ligature.

Oedema develops similarly in the skin of the leg after circulatory arrest under anæsthesia, and a similar fall of protein concentration with time has been observed. The water contents of tissues other than skin also rise, but to a much smaller extent. This does not prove that the response to ischæmia of blood vessels in different tissues varies, since inequalities in tissue tension, vascularity and rate of lymphatic drainage, will prevent an equal accumulation of œdema in all tissues. In the leg of a $1\frac{1}{2}$ kg. rabbit, after occlusion for 16 hours, between 10 and 30 c.c. of œdema fluid, containing from 3% to 5% protein, largely albumen, accumulate in the skin within two hours, and general disturbances develop.

Dye injection. Samples of œdema fluid contain albumen and globulin, and commonly clot within a few minutes of withdrawal if undiluted. It is presumed that its protein is derived from the blood plasma, owing to capillary permeability to protein. It is, however, possible that this protein might be liberated from skin cells as a result of ischæmia and might draw a protein-free fluid from the vessels by osmotic forces.

Abnormal capillary permeability after ischæmia can, however, also be demonstrated by the use of dyes. In three experiments, one ear has been occluded for 24 hours at room temperature, and released immediately after the intravenous injection of Evans Blue (12 mg. in 2.4% solution) into the normal ear. The affected ear becomes blue distal to the site of the ligature, and samples of œdema fluid from it are deeply coloured. The normal ear shows no blue colouration, even though at the base it is thicker than the tip of the œdematous ear.

In a further experiment, dye injected intravenously into the normal ear an hour after release of an occluded ear was found in the œdema fluid an hour later, indicating that the capillary permeability is persistent.

The blue colour develops in the affected ear at and distal to the site of the ligature. After about two hours œdema can be detected proximal to the site and a blue extension along the ear can be seen in this region, the extension being greatest in the midline of the ear.

Discussion.

It is evident that, after a long occlusion, the capillary permeability to protein is at first high, assuming the œdema protein to be predominantly of vascular origin. The subsequent slow fall in œdema protein concentration might be due to one of two causes. If œdema is being rapidly removed from the ear by the lymphatics, samples will resemble the œdema which is being formed at the time, and a falling protein concentration would imply a similarly falling permeability. If, on the other hand, œdema is not escaping from the ear, the fall may be due to dilution by protein-free fluid drawn from the capillaries by osmotic forces to dilute the protein rich fluid which surrounds them. In this case, the vessels might be completely permeable to protein for rather under an hour, and subsequently impermeable.

It is likely that both causes operate. Oedema found proximal to the site of ligature has a lower protein content than that distally, suggesting that it has been increased and diluted by fluid drawn out from normal vessels. At the same time, an estimate of the total amount of oedema protein in the ear can be obtained by multiplying a measure of the amount of oedema by the protein concentration in this oedema. It is then found that the amount of protein may increase steadily in the hours following release. Since some will also escape from the ear, it is evident that the capillaries are still permeable to protein after the first hour, and that dilution is not the only cause for a falling protein concentration.

It is unlikely that the necrosis is a consequence of the oedema. It might appear that a sufficiently severe oedema resulted in vesicles, exudation, drying of the ear, and final separation, and it is certainly true that an area of ear on which vesicles form will usually separate. But some ears which ultimately recover at first develop massive oedema, the ear being thickened 6-fold; and a few which finally separate develop little oedema—in one instance thickening the ear only by 50%. These illustrate wide individual variations from the average. But also the average amount of oedema formed after occlusion for 18 hours at 88° F. is no greater than after similar occlusion at room temperature, although necrosis is rare in the latter case and constant in the former.

The mechanism of gangrene has not been determined, and routine histological examinations have not been made. It is clear from the raised temperature of the ears that the circulation is re-established for the first few days. The falling protein concentration of the oedema fluid indicates that the capillary permeability to protein recovers towards normal. Gangrene might result, either from later thrombosis, from plugging of the vessels by extreme local plasma loss, or from irreversible damage to the skin cells despite a survival of the blood vessels. Further evidence would be valuable on the resistance of different types of tissue to ischæmia and the mode of tissue death in different cases.

SUMMARY.

1. If the circulation to the rabbit's ear is arrested for 18 hours and then released, massive oedema develops within 2 hours.
2. The oedema may subside in a few weeks, or, when ischæmia has been at a higher temperature or has been of longer duration, it may terminate in dry gangrene.
3. The protein concentration in the oedema fluid is initially about 5% but falls progressively.

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SWELLING OF THE HUMAN LIMBS IN RESPONSE TO IMMERSION IN COLD WATER.

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Two years ago while weeding a chalk stream, a task involving tearing the weed by hand from 6 to 12 inches below water for a period exceeding two hours, I noticed that on finishing the job my hands were swollen. I was much interested in their appearance and in the obvious pitting, which I could induce on pressing firmly on the dorsum of the hand, and in my inability to flex the fingers fully either actively or passively. The temperature of the water in the stream was about 12°C .. A year later when weeding the stream again I deliberately worked with the left hand only under water; though the right hand also worked and was always wet, it was not cooled nearly to the degree that was the left. On this occasion the water was at 15°C . and my son worked with me under exactly similar conditions. At the end of two hours our left hands were both distinctly swollen; our right hands showed little obvious change.

This preliminary observation led me to test the effects of cold more deliberately. Having ascertained that swelling could be induced by immersion in cold water in three subjects, subsequent analysis was confined mainly to the reactions of my own hands.

The hand after hanging in water at 5°C . for 2 hours.

In routine observations it has been usual to immerse the dependent hand in a bowl of water kept, by floating ice, at a temperature of 5°C .. The hand has been covered by a loosely fitting thin rubber glove, so that it has remained dry throughout; this to avoid any imbibition of water by the horny layer of the skin, except in special observations in which this was desired and which will be referred to later. On a number of occasions thermal junctions have been attached to the skin of the fingers and of the dorsum of the hand, and readings taken throughout the observation. The glove gives a little protection from the coldness of the water, the temperature of the skin of fingers falling soon, however, to within 1 or 2°C . of the bath and of the skin of the dorsum of the hand to within 2 or 3°C . of the bath.

* Work undertaken with the aid of the Medical Research Council.

When the whole hand is immersed fluctuations of the skin temperature of significant degree do not occur; the temperatures fall to their lowest points and remain there.

The hand that is withdrawn from water at 5°C., where it has rested for 2 hours, is brightly and deeply reddened over its whole surface. It appears to be distinctly and uniformly, though not greatly, swollen; the fingers especially appear to be thicker. All the smaller, and many of the larger, sulci on the dorsal surface of hand and fingers, become less distinct or are lost; though the folds of skin over the extended proximal interphalangeal joints may be unduly prominent. The tendons of the fully extended hand are concealed and cannot be brought into usual prominence. If the dorsal skin is pushed together, the usual fine creasing does not appear, but the skin is thrown into a few coarse folds. At the same time it is obvious that the skin lacks usual mobility; it is resistant to movement; it is less easy to pick up a fold of skin; and these folds are of obviously increased thickness, as are the webs between the fingers. But if a fold of skin is firmly squeezed for a few seconds, the skin is left with a distinct and relatively sharp indentation, and around this occurs a little rampart where the skin is raised and anæmic; it is clear in such that fluid has been displaced in the skin by pressure, in fact that the skin itself is œdematous. Simple firm pressure over the dorsal surface of hand or of the middle phalanx often gives distinct pitting of a more usual kind, and this may be very obvious after immersion for 3 or more hours; it is due to subcutaneous œdema. Flexion of the fingers (active or passive) is hampered, so that the tips of the fingers are brought with less ease into contact with the palm. When the proximal phalanx is kept extended, flexion of the last two phalanges often cannot be completed. Movements of flexion or extension whiten the skin locally more than is normal.

It is clear from these appearances that both in the skin and in the subcutaneous tissues, intercellular fluid is in excess. It is in fact this combination of two separately indentifiable œdemas that gives the hand its peculiar appearance and feel at the end of 2 to 3 hours cooling.

Recovery is slow. The changes described can be identified long after the hand has been dried and has become warm again; they are declining but traces of them remain for 3 hours and sometimes for several more hours.

On removing the hand from the cold water, numbness of its skin is everywhere conspicuous,* and its actions are too clumsy to be effective;

* This numbness results from the action of cold upon nerve endings rather than upon nerve trunks. If skin is cooled to a point as low as 2° to 12°C., numbness is detectable in approximately 2 to 10 min., the onset of numbness being correspondingly quicker at lower temperatures. At the lower temperatures touch sense is lost in about 20 min., but at the higher temperature it is retained, though decreased, for much longer periods. Impairment of pain sense is less and is longer delayed than impairment of touch. If cooling is effected by applying a cold metal surface to the skin for short periods, numbness develops precisely over the area of cooling, it does not extend distally to the area cooled, as it would if cutaneous nerves of any size were responsible. Cooling of the region of the ulnar nerve at the elbow will ultimately produce numbness of the ulnar area of the hand, but this does not occur until long after the cooled area of skin at the elbow has become numb. The recovery of cutaneous numbness produced by local cooling is prompt on warming the skin.

thus any action requiring fine control, such as writing, is impossible. Pins and needles is often to be induced in the appropriate territory by stroking or lightly tapping the wrist over the radial, median, or ulnar nerve. During recovery superficial soreness of the skin is the rule, and this may last for 24 or even more hours; occasionally much pain is experienced.

Enough observations have been made to show very similar effects in the foot after immersing it in water at 5°C. for an hour and more; but observation has been mainly upon the hand, which from many points of view is the more convenient to use.

Volume of hand after exposure to different temperatures.

An early enquiry explored the range of temperature over which swelling of the hand is produced and particularly attempted to find the least degree of cooling that is effective. For this purpose the volume of the hand was measured by displacement, by a method similar to that used by Smirk (7). All observations were undertaken at a room temperature of between 15° and 17°C., during winter months and, at the beginning of each observation it was noted that the hands and feet of the subject were cool and never warm (temperature of hand and fingers averaging about 20 to 25°). An observation upon a hand was always followed by at least 24 hours rest. A thin loosely fitting rubber glove was used to keep the hand dry; and the hand hung by the side of the sitting subject for one hour at each temperature. In most instances two separate volume measures were taken from the hand before and two directly after its period of immersion; actually one measure before and after would have sufficed, as there was never more than 2 or 3 c.c. variation on simple repetition. Each measure was taken as follows:—the hand was withdrawn from its bath and its glove peeled off; it was held aloft for a minute and, the fist being clenched, the circulation to and from the arm was stopped by throwing a pressure of 200 mm. Hg from a reservoir into a pneumatic cuff embracing the elbow joint. The volume of the hand was then measured by water displacement, up to a constant marked line, maintained from day to day just above the wrist. The measurements were made for me by my assistant Mr. A. J. Honour. It was pleasing to find that under this procedure the volume of the hand measured to the mark, varied rarely by more than a few c.c. from day to day; thus, as the volume of the hand was in the average 430 c.c. (left) and 435 c.c. (right), the error in measurement rarely equalled 1%. These results are shown in Fig. 1; the increase in volume being charted in c.c. as ordinates and the temperatures of immersion as abscissæ. The white circles represent measurement from the right and the black circles from the left hand. It will be seen that the volume increase of the hand after an hour's immersion at 5°C. is considerable, amounting to 25 c.c. or approximately 6%. It will be apparent from the chart that this effect on the hand volume declines as the bath temperature rises, until a temperature about 20°C. is reached.

There is, however, no temperature at which the hand is maintained in the dependent position without its subsequently showing a rise of volume. The rise at 20° is very small; but as bath temperature is raised from this level the hand volumes increase, as is shown up to $35^{\circ}\text{C}.$ Briefly, the hand volume is least about $20^{\circ}\text{C}.$, and increases as the temperatures fall appreciably below or appreciably above this level. It is natural to suspect that the two rises of volume illustrated in this curve, are brought about differently; good reason is found ultimately for this belief.

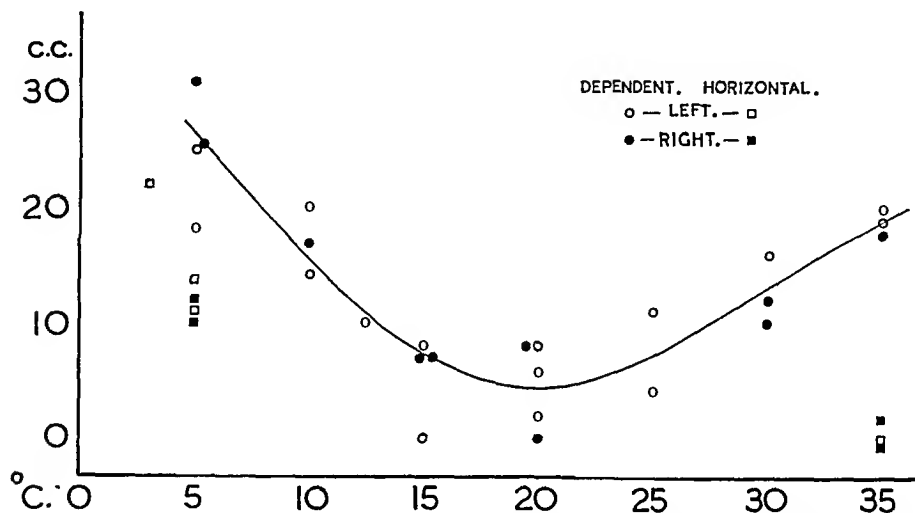


Fig. 1. The ordinates show the amount of swelling (in c.c.) of the hand maintained at the temperatures shown by the abscissæ. The hand was protected from the water bath by a thin rubber glove, and the immersion was for one hour, in each of these observations. Half the observations were upon the right (black) and half upon the left hand (white). Most of the observations (circles) were upon the arm hanging vertically in the bath, and to these the curve corresponds; a few observations at 35° and at 5° , and a single observation at 3° , are charted (squares) in which the hand was immersed with the arm horizontal. Both right and left hand may be assumed to be of approximately 430 c.c. volume, in these and all subsequently chartered observations.

Factors concerned in swelling of the extremity.

The factors concerned in the swelling of an uncovered hand hanging in water at different temperatures are several; they are imbibition, increased vascularity, and œdema formation.

Imbibition. As has been shown previously (Lewis and Pickering (6)), when a hand is soaked in water, the horny layer of the skin imbibes fluid. This imbibition of water by the horny layer is most easily detected in the margins of skin at the base of the nails; the skin margin, ordinarily tough, becomes swollen and spongy. When imbibition is advanced the swelling of the superficial layer of the skin is universal and obvious, the ridges acquiring increased prominence and the skin, especially of the pulps of the

fingers, being thrown into crinkles. The skin of a hand soaked in water at 35° for a half hour becomes opaque and very definitely crinkled, and as the immersion continues this opacity and crinkling increase; these seem at their height after soaking has lasted for about an hour and a half, or a little longer.

The amount of water imbibed in such observations has been ascertained by immersing the two hands simultaneously, the one gloved and the other ungloved, and comparing their volumes subsequently. In these observations the arms have been kept horizontal, to reduce the amount of swelling from causes other than imbibition to a minimum. At 35° the hand imbibes about 8 to 10 c.c. of water in the first hour; subsequently the rate of increase is lower and the full effect is reached at about 1½ to 2 hours, when the increase is about 12-15 c.c. over the original volume (Fig. 2). Ten c.c. of fluid,

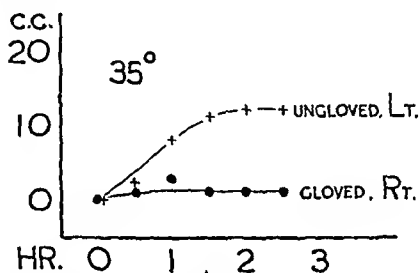


Fig. 2. The left (ungloved) and the right (gloved) hand were immersed in a bath at 35°, the arms being horizontal. The swelling of the left hand, charted in c.c. over a period of 2½ hrs., illustrates the rate of imbibition of water by the skin at this temperature.

regarded as spread uniformly over a hand having a surface area of 350 sq. c. would be equivalent to an addition of about ⅓ mm. to the thickness of the skin.

The amount of water imbibed by the skin at lower temperatures is less. This relation to temperature is well known for imbibition in general and is confirmed by direct observations on skin (6). After a hand has been soaked for an hour at 35° in tap water, crinkling of the skin is very conspicuous, especially on the palmar surface; the skin too has an unusual whiteness or surface opacity; and the free skin margin at the base of the nails is obviously swollen and spongy. After the hand has been soaked at 20°, these appearances are far less conspicuous; the finger tips, however, may show distinct crinkling. After the hand has been soaked at 5° crinkling is never seen, there is little or no surface opacity, and the free margin of skin at the base of the nails is little softened; it is quite clear therefore, that at this temperature imbibition is slight. By measuring it is easy to show that the amount of water imbibed is greater at 35° than at 20°; thus, while in several observations the amount imbibed in an hour at 35° was consistently about 8 to 10 c.c., at 20° it was about 4 or 5 c.c.. It has not been found possible to make any accurate measure of imbibition at 5°. If a gloved and ungloved hand are immersed at this temperature, the latter shows at the end of an

hour swelling exceeding that of the ungloved by as much as 10 c.c.. The appearance of the skin itself conclusively shows that this increase of volume cannot be imbibition. It is in fact due to unusual exposure of the hand. If thermoelectric junctions are fixed to the skin of the two hands, and both are immersed at 5° , the skin of the gloved hand eventually shows a temperature of about 2° above that of the ungloved. This difference in the degree of cooling is sufficient to account for the extra 10 c.c. of swelling of the ungloved hand.

Cooling a gloved hand in a bath at 3° is equivalent to cooling an ungloved hand at 5° ; such an observation at 3° is charted in Fig 1; and it will be seen that the amount of swelling (22 c.c.) exceeds all other charted points (black and white squares) at 5° , and in the average by about 10 c.c..

We may say therefore without hesitation that when the hand swells when immersed in very cold water, the imbibition of water plays no appreciable part in the swelling.

A comparison has been made between the amounts of water imbibed when the hand is soaked in sea water and in tap water, both at $35^{\circ}\text{C}.$. As might be expected sea water gives distinctly less imbibition.

Increased vascularity. When the hand is allowed to hang down, blood immediately collects in it under the increased hydrostatic pressure; the veins are actually seen to swell. It is to be noted however that in the procedure used to measure hand volume it is sought to reduce the blood content by first raising the hand above the head for a minute with hand clenched, and then stopping any bloodflow to the limb, by pneumatic pressure thrown on the upper arm until after the measure of volume is completed. Thus, although the observation is upon a hand that has been exposed for a long time in the dependent position, the actual measure is of a hand that has recently been held aloft and drained. This drainage has for its chief object emptying of the veins; and they are seen to collapse before the measure is made. The veins being well distended while lying in a warm bath and relatively empty while lying in a cold one, the procedure tends to eliminate a varying venous content from the measurements. The amount of blood drained away is found to be about 2 c.c. to 5 c.c. after the hand has been in water at 5° and as much as 7 to 12 c.c. when it has been in water at 35° .

The procedure cannot be said completely to eliminate the content of the veins or of vessels of smaller order; for it is not merely immediate response to hydrostatic pressure that affects our measurements. Drury and Jones (1) found that when an obstructing pressure is thrown upon the veins of the arm kept at a given temperature, they do not reach their full size as the pressure rises in them to its maximal point. They continue to expand as their tone relaxes during a period of many minutes. We must assume that small veins and venules of the limb behave similarly. When the arm remains dependent, its vessels, on the venous side, will increase in capacity owing to loss of tone; and this increase will be greater when the arm is kept warm than when it is kept cold. We may regard it as highly probable,

when the arm is drained by lifting it, that more blood will be retained in it when its vessels are in lower than when they are in higher tone.

When the hand hangs still in water at 35° , the pressure in its veins is equal to about 40 mm. Hg.* In such circumstances, as Drury and Jones (1) and also Smirk (7) have shown for the foot or hand, œdema will form in a hand (say of 400 c.c.) at a rate of about 4 or 5 c.c. in a half hour. The increase in the dependent hand at 35° is about 12 to 15 c.c. in the first half hour, and subsequent increments agree sufficiently with previous measurements for œdema itself. It would seem likely therefore that about 10 c.c. of the total volume increase in the first half hour is blood and the remainder is œdema. This necessarily approximate calculation receives some confirmation; if the circulation to the arm is kept completely obstructed for the first 20 mins of its dependency, if it is then released, and if the hand volume is taken at the half hour, the measure must contain much less œdema than the usual measure; nevertheless it shows a volume increase of 10 c.c., most of which I conclude to be blood. The rate at which the volume increases as time passes is to be seen in the left hand curve of Fig. 3; the rate of increase rapidly declines. It is probable from Drury and Jones' work that blood content rises chiefly in the early period of dependency and that subsequently œdema is steadily added.

We may certainly conclude that swelling of the hand held dependent at 35°C. is due in part to increased blood content and in part to transudation; the precise relation of the two has not been ascertained, but it is roughly calculated that in the first hour of accumulation the blood and tissue fluid will be about equal; blood accumulation being limited to the early period, and transudate being a steadier and continuing contribution.

An estimate of blood contributed to the swelling of the hand in water at 5° is more difficult to obtain, but it is almost certainly smaller than that just discussed. Since all the main vessels of the hand are in a state of contraction in response to cold we might be inclined to ignore this factor; but we should not be quite justified in doing so knowing that water at 5°C. applied to a *finger* causes dilatation of its arterioles and increases bloodflow through it (Lewis (3)). Now, when the *hand* is immersed at 5° , no evidence of increased bloodflow is to be obtained; the temperature of the fingers remains steadily a few degrees above bath temperature. Presumably, though dilatation of arterioles happens, the vessels feeding these are contracted and as I have shown in another connection (4), do not permit an appreciably enhanced bloodflow. The expansion of the arterioles, however, and the visible dilatation of the minute venules of the skin in response to the cold, must be acknowledged as providing an increased blood reservoir; to be set off against this is the obviously contracted state of arteries and veins. Thus it is probable that the increase in the size of the hand occurring at 5°

* When the hand hangs down and is kept in persistent movement, as in gripping and relaxing, the average venous pressure will be much less. In these circumstances I have found the amount of swelling at 35° for 1 hour to be in the average half that for the still hand.

is not largely contributed to by blood. Certainly there can be no more than a preliminary contribution from this source, since the dilatation of small vessels, which is known to occur, soon comes to a maximal point and then waxes and wanes about a lower level.

This preliminary consideration of vascular content brings us to the main contribution, which is that of œdema.

Oedema. Looking at the amount of swelling displayed to right and left in Fig. 1, we see that it is not very dissimilar at 5° and 35° . This curve, it is to be remembered, is composed entirely of tests lasting each for 1 hour. The swelling that happens at these two temperatures is different in mechanism.

A first indication of this difference is found in the dissimilarity of the skins; the cooled skin becomes relatively immobile and thick; the warm skin remains supple.

If the volumes are measured at $\frac{1}{2}$ hour intervals and the corresponding curves are charted, they are as shown in Fig. 3. At 35° the curve, starting

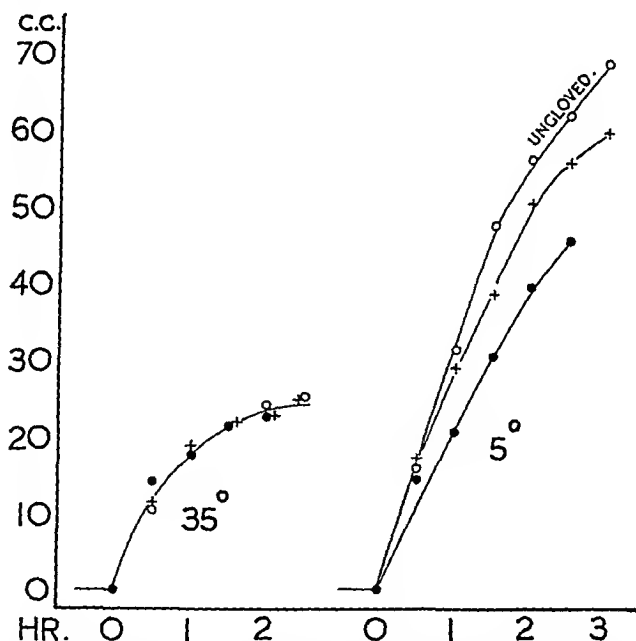


Fig. 3. The left hand curve shows the swelling of the hand, in three separate observations, hanging down in water at 35° for 2 to $2\frac{1}{2}$ hrs.. The rise in volume represents in part an increase in blood volume and in part an increase in tissue fluid.

The right hand curve shows three examples of swelling of the hand hanging down in water at 5° for a similar time. The rise in volume represents chiefly an inflammatory exudate.

The hands were in rubber gloves in all observations except the one marked ungloved.

rather steeply, moves quickly to a shoulder and the ascent then continues very slowly. Most of the increase occurs in fact within the first hour and much of this is vascular. At 5° the curve begins steeply, and it continues

to rise along much the same slope for 2 hours or longer. Thus the swelling soon surpasses greatly that occurring at 35°. At the end of the 2nd hour, swelling is happening in both hands and in both it is the result of increase in tissue fluid; but the rate of swelling is in the one case about 20 c.c. per hour and in the other about 2 c.c. per hour. This is a first and striking difference.

Another difference between the behaviour of the limb at 35° and 5° concerns posture. The swelling of the limb in the 35° bath is determined by dependency. If the limb is kept horizontal it remains without change of size. Whether we consider the swelling from the standpoint of blood content increase or of œdema, increased venous pressure is essential to it. Oedema forming at 35° is a simple transudate. Oedema at 5° occurs differently.

Oedema from cold.

This œdema, as has been seen, is poured out rapidly, and for several hours almost uniformly. So much fluid appears that distinct pitting can be obtained within 3 hours. In this time 60 c.c. or more may collect in a hand of 430 c.c. volume, an increase of about 15%. If the hand is withdrawn from the cold bath, and is allowed to warm again, its volume is less within a half-hour, showing that the output of fluid into the tissue spaces soon ceases. An increase of volume after withdrawal from the bath has not been observed.

The production of œdema from cold, unlike that occurring in the warm, occurs even though the arm is kept horizontal. The rate of œdema formation is lessened by this posture however. Thus in four comparisons at 5° the increase of volume within an hour averaged 13 c.c. with the arm horizontal, and 26 c.c. with the arm dependent (see Fig. 1). Although the swelling of the hand is less, the condition of the skin is not so different; a fact suggesting that it is the œdema of the subcutaneous tissue which is chiefly affected by the postural difference. In harmony with what has been related is the fact that, if the hand hangs down in the water at 5° and a pressure of 60 mm. Hg is imposed on the veins of the upper arm, the rate at which the volume increases is very definitely raised (Fig. 4).

The rate at which œdema forms under the influence of cold and its occurrence when venous pressure is unraised, suggests that this œdema comes primarily from an increased permeability of the endothelial walls of the vessels.

Character of the fluid. In the hand swollen after hanging in water at 5° for two hours, a few cubic millimetres of clear fluid can be expressed or sucked into a needle passed into the subcutaneous tissues, just beneath the skin. This is diluted in a capillary tube with nine parts of saline, and to the whole an equal volume of absolute alcohol is added. This gives, when thoroughly mixed, a fine precipitate the opalescence of which is compared, as is later the centrifuged deposit, with that obtained when chosen dilutions of the subject's blood serum are similarly treated. The estimate is of course

an approximate one, but it has been clear in three separate experiments that the œdema fluid contains less than a half, and more than a third, of the protein contained in the serum (itself measured to contain 7.93% protein). The estimated content in the œdema fluid therefore is approximately 3%, a value much above that found for simple subcutaneous transudates.

Thus, the view is confirmed that the fluid is not a simple transudate and that increased permeability of vessel wall is present.*

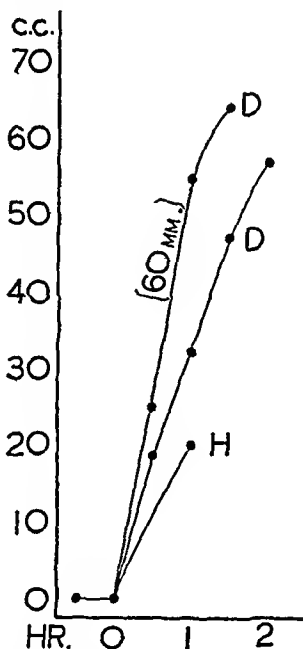


Fig. 4. A chart illustrating the rates of swelling of the hand immersed in water at 5°. The three observations compared, indicate the rate of swelling, with the arm horizontal (H) dependent (D) and dependent with a 60 mm. obstructing pressure on the veins of the upper arm. In all three observations the hand was ungloved.

Injury by cold.

Facts known to us suggest that cold of certain intensities directly injures the superficial tissues in man. I shall do no more than refer to the sequels of very prolonged cooling, for example chilblain-like states. The relation

* When an urticarial wheal is produced on the skin this presents the phenomena of inflammation and the fluid passing into the tissue spaces has a protein content not far below that of blood serum (Lewis (2)). The method now used has been described in this earlier paper.

It is interesting to note the effect of increased venous pressure (60 or 70 mm Hg) upon the formation of such wheals. They tend to be reduced. In that case we are dealing with the passage out from the vessels of almost all the fluid that enters them; therefore, any interference with the bloodflow to the skin tends to diminish the amount of fluid available for the wheal. Where, as in the response to cold, the increase of permeability is less, the furtherance of œdema formation by increased venous pressure can be understood. There is no real inconsistency between the different effects of increased venous pressure in the two instances.

of such conditions to cold has been reviewed recently (5); but the observations of Smith, Ritchie and Dawson (8) should be noted especially, for they clearly demonstrated that very prolonged action of cold, of a degree less than that required to freeze, causes œdema and clear evidences of inflammation in the rabbit's foot. From a theoretical standpoint it would seem more convincing to be able to establish that cold is followed by evidence of tissue injury when it has acted for relatively short periods of time. It is especially desirable that the relation of cold to injury should be understood in man.

There is in general a close relation between injurious and painful stimuli of the skin; painful stimuli seem always to be followed by visible evidences of cutaneous injury, if these are carefully sought. Cold is no exception. If the hand is immersed and maintained in water at 25° or 20° the sensation experienced is one of pure cold. But if the hand is similarly held in water at 5°, in addition to the sense of cold, pain is very definitely added. As the temperature scale is descended this sense of pain begins to be recognised at 15° or perhaps a little above this temperature (observations upon 3 subjects). It is a temperature to be remembered because other evidences of injury appear at about the same level.

When a finger is immersed in ice-cold water, pain in it shortly becomes severe enough to be disagreeable; this pain continues during immersion for 5, 10 or more minutes and it then lessens. The finger becomes comfortable again, and observation shows that its temperature rises. There is a reaction in the finger, resulting in vasodilatation. This vasodilatation has been shown to depend upon an axon reflex; although it continues after section of the cutaneous nerves, it is lost when these nerves degenerate (3). But a similar axon reflex is a feature of the response to very many if not all forms of cutaneous injury. Thus, its presence as a response to cold definitely suggests that the latter injures; and the suggestion is the stronger because the response to cold can be recognised not only at 0° but readily at 5° and 10°, and with care at a point as high as 15° or even occasionally at 18°.

In the present observations new evidence that the skin and subcutaneous tissues are damaged by severe cold has been found in the œdema described; the fluid collecting has the protein content of an inflammatory œdema. It is also noteworthy that this œdema in response to cold begins to appear at 15°C. or a little above this point (Fig. 1). It will be remarked that in exposures of 1 hour's duration, such as are comprised in this graph, the nature of the swelling is known to be œdematous with greater certainty for the 5° than for the 15° reaction; but it is beyond doubt that œdema occurs at the higher temperature; for actual pitting appears in long exposures at 15°.

There is other evidence, previously recorded (3), that cold injures the skin, for example the appearance of cutaneous redness that persists after the skin has been rewarmed, but the observations here collected are in particular harmony with each other owing to the similar range of temperature over which they are displayed.

It is presumed that the œdema of the skin in response to cold as here described results from the liberation of H-substance, the mechanism believed to be common to cutaneous injury. But our knowledge of H-substance released by injury is almost confined to skin itself. It is therefore important, to show that subcutaneous œdema can be produced similarly. Common experience tells us that it can be caused by injury. If histamine (1 c.c. of 1 in 30,000 base) is injected into the subcutaneous tissues of the forearm, swelling becomes identifiable within a few minutes. If several such injections are made within a few centimetres of each other, the region of the forearm becomes boggy and distinct deep pitting occurs on firm pressure.

If 60 or more c.c. of albuminous fluid may be lost to the hand exposed to cold for 3 hours, it is clear that much larger quantities will be lost when larger parts of several limbs are exposed similarly for many hours or even days, as when sailors are adrift in open and swamped boats. The loss of such amounts of fluid should be taken into account, as should the protein loss, in estimating the effects of exposure to cold upon the circulation as a whole.

CONCLUSIONS.

1. When an extremity is cooled, as by immersion in cold water (5°C.), it swells. This increase of volume occurs in both skin and subcutaneous tissue, and may amount within 3 hours to as much as 15% of the original volume.

2. The swelling is due mainly to an œdema of the tissues, judged to be inflammatory from its relatively rapid outpouring and from its relatively high protein content. The contribution in the form of imbibed water is very slight.

3. From this and correlated evidence it seems that cold directly injures the skin and subcutaneous tissues. This effect begins at about 15° to 18°C. and increases as the scale of temperature is descended.

4. The production of œdema as a result of long continued immersion of the limbs in cold water should be taken into account, as should protein loss, in estimating the effects of exposure to cold upon the whole circulation.

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TROUSSEAU'S PHENOMENON IN TETANY.

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TROUSSEAU described (9, 10) his chance discovery of a phenomenon, namely, the artificial production of tetanic cramp of the hand, in a patient suffering from tetany who was being bled with the assistance of a bandage applied around the arm. He believed it could be provoked when the arterial or venous circulation to the arm was interrupted, when the median nerve was compressed in the arm or the brachial plexus above the clavicle, when the femoral artery was compressed, or a ligature tied around the thigh. He sums up that it "is effected by simply compressing the affected parts, either in the direction of their principle nerve-trunks, or over their bloodvessels, so as to impede the venous or arterial circulation."

The mechanism of its production has been much debated, Kussmaul (6) emphasised arterial compression, but most writers (4) emphasise pressure on nerves. Hoffmann (5) ascribed it to peripheral anæmia because he found incomplete or intermittent occlusion to be ineffective. Weiss (11) believed that it results from compression of sympathetic fibres in the wall of the vessel. There is recurring uncertainty in that compression of a limb is calculated to affect both artery and nerve; and, while for the most part the observations cannot be held to discriminate, there is difficulty in attributing the effect to the appropriate structure. Frankl-Hochwart (4) says that it cannot be simply vascular, since Müller (8) has produced it in the foot by compressing the peroneal nerve, which has no large vessel beside it; and he himself found that in a thyroidectomised dog, the artery and nerves being laid bare, strong compression of the artery was without effect, while very slight interference with the nerve produced tonic cramp promptly (3 and 4). Moreover he found in man that compression of structures in one bicipital groove may produce cramp in both arms, a fact he states to have been confirmed by others, and which has led to the view that the phenomenon may be reflex.

It is clear that the matter is no simple one, and probable that spasm may be provoked by one of several essentially different procedures. Certain facts are to be noted. The tonic spasm provoked by Trousseau and the

* Work undertaken with the aid of the Medical Research Council.

quick response of the facial muscle to tapping over the facial nerve are distinct and not necessarily related directly to each other. Thus it is not quite relevant to cite increased excitability of nerves to tapping or to electrical excitation (2), in attempting to explain the cramp-like response of the arm to compression. Again, the response to compression of an isolated nerve, for instance the peroneal nerve, does not necessarily arise directly from mechanical stimulation of the nerve; it might arise indirectly by rendering the nerve anæmic. Furthermore compression of a main artery, however powerful, does not usually stop all bloodflow to a limb; failure to produce spasm in this way does not exclude its origin in local anæmia. Thus, the present evidence does not show unequivocally, as has been generally thought (1), that Trousseau's phenomenon is due to stimulation of the nerve by pressure, and that anæmia of the limb plays no part.

These brief introductory remarks will suffice to show it to be important, in attempting to explain the phenomenon to which Trousseau's name is generally applied today, that the chief observations and experiments should concern this precise phenomenon, and should not concern in the first instance what may be regarded as related or similar phenomenon. When the response in each separate procedure (Trousseau, Chvostek, etc.) is explained, we may with greater safety proceed to correlate all in searching for a possible common factor. I here record observations upon one phenomenon only, namely, tetanic cramp of the hand following the application of pneumatic compression of the upper arm, the usual manner in which Trousseau's sign is now elicited.

Observations.

M.H., a woman of 33 years was admitted to hospital, under the care of my colleague, Professor Himsworth, suffering from tetany following partial thyroidectomy. At the time she was seen she was recovering under treatment with calciferol and calcium lactate, there being no spontaneous spasm, though spasm of the arm was still easily induced by compressing it; Chvostek's sign had failed to be elicited for a few days.

On the occasion of her examination, a sphygmomanometer cuff blown to 200 mm. Hg on the upper arm was followed by tetanic spasm of the hand after an interval of time, which presented considerable uniformity, namely 40 to 50 sec.. The first change came as an adduction of the thumb, followed shortly by flexion of the metacarpophalangeal joints with extension of the phalangeal joints; later came flexion at the wrist. The patient complained of tingling in the fingers at or shortly before the onset of spasm. The cuff pressure being released at the end of 2 or 3 min., recovery from spasm always occurred rapidly, so that the hand felt normal to patient and observer within 7 or 10 sec.. The time relations are of interest. The spasm does not occur as an immediate response to compression of the arm, but only after about a minute's delay; this fact at once suggests that the direct effect of pressure is not responsible for the phenomenon. Support for the same idea is found

too in the fact that the time relations remained exactly the same whether 200 or 300 mm. Hg was thrown into the cuff, the pressure being applied abruptly from a reservoir.

To resolve the point as between the direct effect of pressure and the indirect effect through blood supply other tests were used.

Two cuffs, a proximal and a distal, were placed on the upper arm, and the pressure of 200 mm. was thrown into the proximal one only and continued. In due course (55 sec.) spasm began in the hand and developed. At 100 sec. the distal cuff was inflated and the proximal pressure released; all spasm had gone within 15 sec.. After a delay of 5 min. to allow full recovery, the experiment was repeated, reversing the order of inflating the cuff. The pressure was thrown first into the distal cuff. Spasm appeared as usual in the hand; the same pressure was now thrown into the proximal cuff and that in the distal released. This procedure was followed by no trace of recovery. These experiments gave constant results on repetition.

It seems clear from the observations in which the proximal cuff is first distended, that the onset of cramp is due to changes happening within that cuff, rather than below it. Thus it cannot be due to loss of blood supply of muscles below it, or to loss to nerve endings in the muscles; for in either case the effect would be maintained by transferring the obstruction from proximal to distal armlet. Recovery in fact started as promptly as though on the release of the upper cuff the second had not been distended; it was not however always complete.

It seems clear from the observation in which the distal cuff is first distended, that the onset of spasm in the hand is not the result of a direct pressure stimulus on the nerve; for when the pressure in the lower cuff is released, after distending the upper, there is no recovery. Since the pressure imposed by the upper cuff has had no time to act it follows that the spasm is maintained because the change which occurred under the distal cuff and induced the spasm is unaltered; there has been a relief of pressure, but there has been no restoration of blood supply. I conclude therefore that the onset of spasm and its maintenance is due to loss of blood supply to the nerve beneath the cuff.

It is to be noted that loss of blood supply to the nerves of the forearm occurs and is maintained in both forms of experimentation, but that in the first case the blood is restored to the upper part of the nerve originally deprived of its supply, whereas in the second case blood supply is restored to no part. It is now to be said that a definitely longer compression by the lower cuff was required to produce a similar effect to that of the upper cuff (75 to 90 secs in the one case and 45 to 60 secs in the other).

Ischæmia of the nerve is responsible for the spasm known as Troussseau's phenomenon and a shorter period of ischæmia seems to be required for a proximal than for a distal stretch of nerve. An exactly comparable series of effects has been observed for paralysis induced by asphyxiation of the normal limb, as has been shown in a previous publication (7). In both

instances, one irritant and the other paralytic, the effect comes first in distally supplied tissues, the small muscles of the hand, to be followed later by an effect in the forearm. The paper referred to describes in full detail the procedures here used and their significance.

That the spasm results from ischæmia of the nerve where compressed was further confirmed in the following ways. If the special clamp, described in the publication just referred to, was used, the spasm could be induced by throwing a pressure of 70 mm. Hg on the upper arm. The pressure exerted by this clamp is adequate to stop all bloodflow to the subjacent nerves, while leaving the main artery and the venous return unaffected. If the same pressure was thrown into a simple circular cuff on the arm, spasm did not occur; that is so because the venous return is in this circumstance impeded and, as the blood pressure in the minute vessels of the nerves under the cuff is kept high, the blood in these vessels continues to flow.

The effect of ischæmia could also be displayed in another way. If spasm had been induced by cuff pressure, enough to obliterate the main artery of the limb, the recovery from spasm could be greatly delayed by compressing the artery above the cuff and then releasing the cuff pressure; for the digital pressure would now maintain a partial ischæmia in a nerve already suffering from lack of blood supply. Similar digital pressure on the brachial artery at precisely the same point, did not by itself induce spasm of the hand in this patient.

SUMMARY.

In a case of tetany, Trousseau's sign was easily elicited. It has been shown, in this case, that the spasmodic affection of the hand on occluding the circulation to the arm, resulted from ischæmia of the nerves under the cuff. The phenomena of irritation described by Trousseau, and those of paralysis where the nerves of the normal limb are deprived of circulation, are interestingly comparable, though the former are naturally much earlier manifestations.

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OBSERVATIONS UPON THE VASCULAR AXON REFLEX IN
HUMAN SKIN, AS EXHIBITED BY A CASE OF URTICARIA,
WITH REMARKS UPON THE NOCIFENSOR NERVE HYPOTHESIS.

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M.W., a married woman of 26 years, came in June, 1942, complaining of an urticarial eruption. Apart from two attacks of pleurisy in her 23rd and 25th year her health had always been good, and from these attacks she was fully recovered. There was no history or family history of eczema, asthma, or hay fever.

In February, 1942, news reached her that her husband, who was in the army, had been missing since the loss of Hongkong Island; this news very naturally gave her much worry. In April the eruption came and afterwards continued. No material change of habits could be recognised; she had continued to care for her two children, aged 6 and 4 years, without change of abode or diet.

The rash appeared suddenly; she found it all over her trunk, limbs and face on waking one morning. It came nearly every night while she was in bed, and was found in the morning chiefly on the thighs and lower abdomen; but few parts of her body were exempt. During the day time she was comparatively free, though spots continued to appear here and there and were sometimes numerous. She thought the rash to be related partly to menstruation, being at its height, so she at first said, for a few days before and for a few days afterwards; actually she could not be sure of the precise relation, and under observation none could be established definitely. She stated the rash to be provoked easily by hot baths, which she had therefore given up taking; the heat from a fire would also bring it. She ascribed the attacks at night to becoming over-warm in bed. She had observed no relation to exercise, but strenuous exercise was rarely taken.

In the attack the region of her eyes had become swollen a score of times, not sufficiently to interfere with vision but enough to be very disfiguring; the swelling was nearly always symmetrical, but once it was confined to the left eye. In at least 10 attacks her lips had swelled, upper and lower, and

* Work undertaken with the aid of the Medical Research Council.

sometimes to twice their usual thickness. Twice her tongue had become swollen on the left side to twice its usual thickness, rendering her speech unintelligible and giving much discomfort. These swellings, which also attacked the fingers, usually accompanied the thick rash on the body and were discovered on waking; they would last an hour or two and leave no soreness or bruising behind.

On examination the woman was found to be well nourished (142 lbs.) and unemotional; her colour was normal out of the attacks; the heart and blood pressure were normal; her lungs presented no physical signs, the chest X-ray was normal; her urinary functions were normal; no signs of infection in throat, sinuses, pelvis, or elsewhere could be discovered with the exception of two carious teeth. Temperature, pulse rate, sedimentation rate, and blood count were all normal; the complement fixation test was negative.

Observations.

Spontaneous attacks. There were but few days in a month of close observations on which the skin remained quite clear of eruption; usually in the day time a few urticarial wheals a few millimetres or a centimetre in diameter, and each surrounded by a bright red flare, could be found scattered over trunk and limbs, and in other places small red stains, the marks of subsiding eruption. They were seen from time to time on all parts of the body except the palms of the hands and soles of the feet. Large wheals subsided in the space of an hour or more, leaving a red stain of several or many hours duration behind them. In what she called attacks the eruption was abundant, the wheals often becoming confluent to form irregular patches of raised skin; the lower abdomen and thighs were particularly involved, and her statement that they came mainly in the night and almost every night was amply confirmed. In such attack it was the rule for her face as a whole to become conspicuously engorged with blood, and this was so with or without wheals on the face and with or without swelling of the eyelids. There was no perceptible increase of sweating in the attacks. Sweating under artificial heating was normal.

Relation to heat. To confirm her statement she was put to sit in a bath of water, as hot as she could tolerate without discomfort ($43^{\circ}\text{C}.$); she remained in it for 20 minutes. Her face was then strongly engorged, and much of the skin of thighs, legs, lower abdomen and back, parts that had been submerged, was covered with closely set or confluent wheals. Isolated fresh wheals had also appeared on the skin of trunk and arms. This test was done on two occasions. On two other occasions her legs were placed in water at $45^{\circ}\text{C}.$ At 25-28 min. the face was very red and sweating was general. At 30-35 min. scattered small wheals were appearing on the upper limbs and thighs and continued to appear on all the limbs (though not on immersed skin) up to about an hour when the bath was discontinued; no more wheals developed. To test her belief that warmth in bed was

responsible, a bath was given on another day at 38°C. for 20 minutes, the temperature being chosen as the maximal to which her skin could rise in bed. It produced no eruption.

A cylindrical copper box 3.5 cm. in diameter was placed on the skin of the thigh or arm when this was free from rash, and water percolated through it at various temperatures, the surface temperature of the covered skin being recorded thermo-electrically. In all observations care was taken that the box lay lightly on the skin. Temperatures up to 41° were without abnormal effect; but after circulating water at 42.5° for 10 minutes, the area of skin warmed was found to have assumed an unusually strong local hyperæmia with surrounding flare. The skin was unwhealed, but the redness persisted for 2 hours. Temperatures of 43.5 to 45° produced striking effects. Within

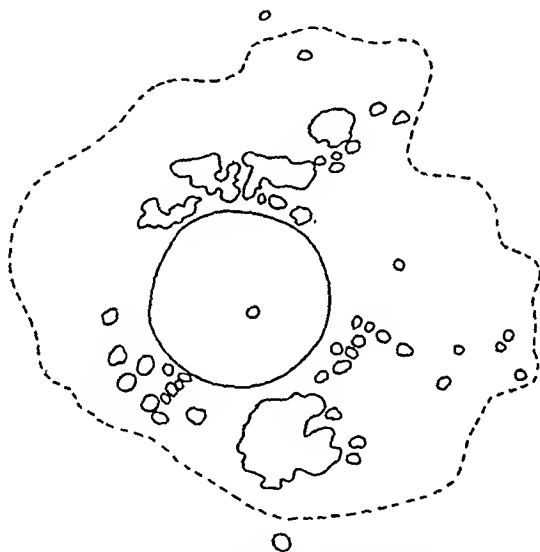


Fig. 1. ($\times \frac{2}{3}$). The large circle encloses with continuous line the area of the right thigh heated to 44°C. for 8 min. The broken line represents the approximate outline of the flare which developed on surrounding skin. Wheals began to appear at the 8th min. and continued to appear and to grow until the 25th min. These wheals, as can be seen, occupy the area of flare; many have become confluent; two stand a little beyond the boundaries to which the flare was marked; one only has come on the heated area itself.

In this and subsequent figures, the top of the figure is proximal. All figures were traced directly onto cellophane from the original ink marks on the skin.

a few minutes of application a vivid flare appeared in the skin around the box and at 7 min. or a little later wheals began to appear. At the lower temperature a few discrete wheals appeared over the surface of contact; but most of the wheals, and exclusively so at the higher temperatures, surrounded the heated area. These satellite wheals would appear on any parts of the skin presenting flare, occurring often as far as 3 or 4 cm., and occasionally as far as 6 cm., beyond the edge of the heated area. They

might continue to appear for as long as 20 minutes after removal of the box and sometimes run together to form confluent patches of swelling (Fig. 1). The relation of the wheals to the area of skin previously showing flare was quite conclusive; they would appear to its outermost margin, and only small isolated wheals, and these very occasionally, appeared in skin a little beyond the margin of the flare as previously defined (Fig. 1). Although the first wheals to be noticed were usually in regions where they ultimately lay thickest, and that was often within 2 cm. of the edge of the box, they did not in general appear first near the box and later farther from it. In the rule scattered wheals appeared in numbers more or less simultaneously, to grow and to be added to later; occasionally the most peripheral wheals were noticed earliest. With the higher temperatures of stimulation, the local redness where the box had been applied was of great intensity and persisted for very many hours.

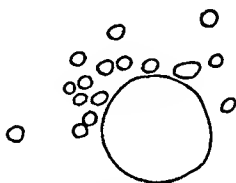


Fig. 2. (Nat. size). The large circle represents an area of forearm skin frozen for 5 sec. with solid CO_2 . The tracing shows the relative position of the main wheal and its 16 satellites.

These tests seemed clearly to show that the natural temperatures of the skin occurring when the patient lay warm in bed were insufficient by themselves to bring wheals to the skin, and that temperatures known to be within the range of producing skin injury were necessary; the temperature required to produce wheals on the skin actually heated was around 43°C .; 45°C . gave no local wheal. Other forms of injurious stimulation were tried.

Freezing. The end of a stick of solid CO_2 (14 mm. diam.) was pressed against the skin for 5 sec.. It produced the usual circular wheal over the whole area frozen in 3 min.. This wheal was surrounded by a flare, within which small satellite wheals began to appear at the 8th minute; these were full in number and size at about the 20th minute and their outlines are shown in Fig. 2.

Faradism. A faradic current was passed from twin platinum electrodes into the skin of the forearm, the current strength being sufficient to bring and maintain local goose skin. By the end of 4 min. when stimulation ended, a widespread and brilliant flare surrounded the point stimulated. At $5\frac{1}{2}$ min. minute wheals were appearing in numbers within and throughout the area of flare, these continued to appear and grew till the 16th min. (Fig. 3). This remarkable constellation of wheals in response to faradism was seen on many occasions, and always in the same form. The satellite

wheals, like those previously described, picked out first the papillæ of the skin and often became confluent subsequently. The points at which they were to come could be recognised by the preliminary appearance of small and unusually bright red spots.

Histamine. The unusual reaction of the skin to three separate forms of local injury led me to use histamine as the stimulus. The strength was 1 in 300 of the base; a drop of this was placed on the skin and a fine needle

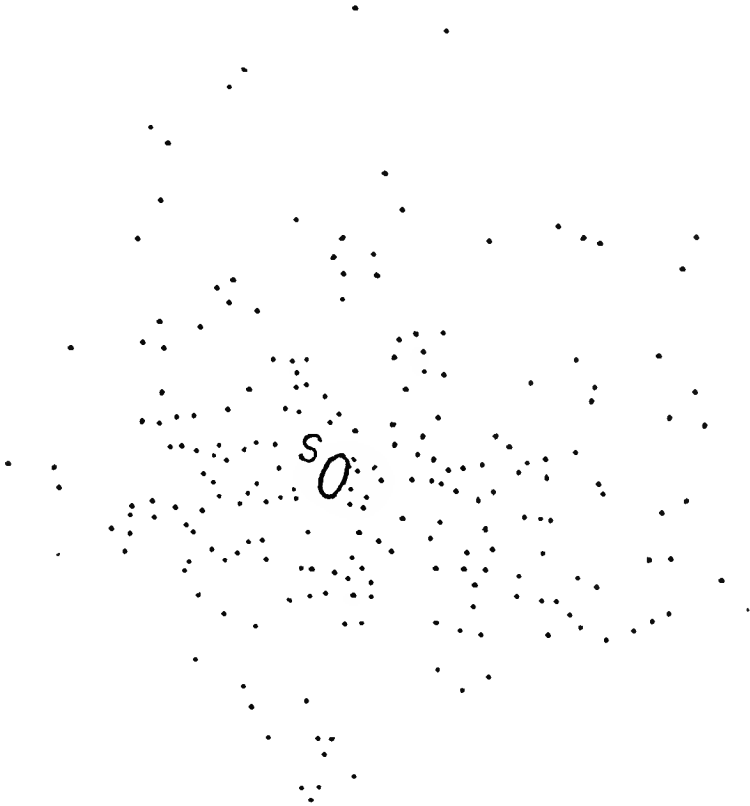


Fig. 3. ($\times \frac{3}{2}$). The forearm skin was stimulated with faradic current and twin electrodes at S. In the tracing the centres of satellite wheals are represented by dots. These wheals appeared more or less simultaneously, and covered with considerable accuracy the area of the flare at its height.

pricked the skin lightly through it at three closely set points. The drop of histamine solution was then carefully removed with filter paper and the reaction watched.

The preliminary reaction was normal, three wheals appearing at the site of the pricks and soon (4th min.) becoming confluent; the usual flare surrounded them. Small satellite wheals started to come about the 7th min.

and continued to appear till about the 12th min.. These satellites were scattered around the histamine wheal, close to it or as far as 3 cm. from it. An example of such a reaction, repeatedly obtained, is given in Fig. 5.

Simple needle pricks gave no more than a small local response and histamine smeared on uninjured skin gave no reaction.

Strokes and pressure. Having found in previous cases of urticaria that simple stroking of the skin during the attacks (see also Harris (4)) often gives whealing, this form of mechanical stimulation was tried. But in this patient it constantly failed, even if the same line was stroked several times in succession. No more than a bright local red reaction and a surrounding flare were obtained.

It was noticed however that if, during other tests, the patient happened to lean with the elbow on the arm of a hard chair for several minutes, a local wheal would appear at the point of pressure; and sometimes the eruption on the thighs in the morning seemed possibly to be associated with lying on the side. The cuff of a sphygmomanometer was placed on the upper arm and pumped to 150 mm. Hg and left in place for 15 min.. On release the usual reactive hyperæmia followed, involving the whole arm from the level of the top of the cuff downwards. In normal subjects the whole of this flush fades simultaneously; but in this subject the flush disappeared first from the arm below the cuff, leaving a pink band of skin where the cuff had been. Soon the redness of this band increased and very many bright red spots, and a few score of scattered wheals appeared within 10 min. of release over the area that had been compressed. It was noticed too that this area of pressure was sharply defined from the rest of the arm by its higher temperature. The events here described were repeatedly observed in separate tests. The increased temperature of the reddened band amounted to as much as 2°C.. A pressure of 300 mm. in the cuff produced a greater effect than did 150 mm.. The wheals though scattered over the compressed skin tended to appear in certain areas more than others, being usually much more numerous on the back than on the front of the arm. They appeared right up to the margins of the area of pressure, but in no instance were wheals seen to occur outside the pressure boundary. Wheals produced in this way are not the result of loss of blood supply to the skin, for the skin below the cuff was from this standpoint equally involved and yet showed no wheals; they result from pressure injury.

When a fold of skin was caught up between finger and thumb and strongly compressed for 12 min. and released, red spots and wheals were appearing widely in the skin surrounding that compressed within 10 minutes, and some minutes after release this compressed skin was covered with small wheals and was 2° to 3° hotter than the unaffected surrounding skin. The satellite wheals extended as far as 4½ cm. from the areas of compression (Fig. 4).

It was at first difficult to see why in the case of cuff pressure no satellites occurred, whereas with a prolonged pinch these were numerous. The

explanation lies in the degree of injury; when a fold of skin is caught, distortion is greater as may be seen by the presence of pitting of the skin, immediately at the release. Severer pressure for a few seconds was likewise efficacious. If the square cut end of a small rod such as the unpointed end of an ordinary pencil is pressed very hard upon the skin over a bone or tendon, a sharply cut mark is left on the skin, though there is no breach of surface; the skin where pressed upon remains depressed, while around this is a little rampart due to displaced tissue fluid. An injury of this kind from pressure exerted for but a few seconds in our patient produced subsequently local whealing, with satellite wheals scattered around for 2 or 3 cm..

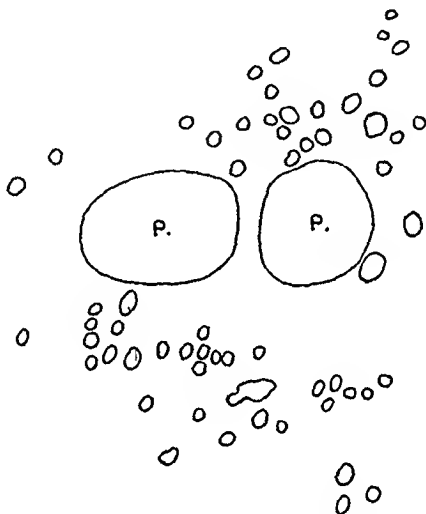


Fig. 4. ($\times \frac{2}{3}$). A fold of the forearm skin was picked up between finger and thumb and compressed as firmly as possible for 12 min.. The areas of pressure and counter-pressure (P) are outlined, with a large number of small and larger satellite wheals. These satellite wheals started to appear about the 10th min.. The areas of pressure began to wheal from the 20th min. onwards, and shortly were completely covered by small wheals.

Although these observations have disclosed an extraordinary reaction of the skin to compression, they have not revealed the main reason for spontaneous eruptions in this patient. Close watching of this patient during the hours of sleep failed to establish a clear relation between the morning eruptions and the part of the body on which pressure had recently been exerted.

Stretching the skin for periods of many minutes produced no wheals, except over the areas held in submitting the intervening skin to tension.

Cooling the skin with ice for 1 min. and rewarming gave a normal reddening of the skin, but no whealing.

THE MECHANISM UNDERLYING SATELLITE WHEELS.

Involvement of nerves.

The close correspondence between the area of skin covered by satellite wheals and that covered by the flare, when the skin of this patient was injured by such agencies as heat, freezing, or faradism, or when histamine was introduced on the point of a needle, itself strongly suggests that these wheals were provoked as is the flare through a local nervous mechanism. The idea has been probed further.

Local anæsthesia. The satellite wheals could be shown to depend as does the flare upon the activity of local nerves. A small area of skin (Fig. 5 An) was anæsthetised by intradermal injection of 0.03 c.c. of 2% novocaine.

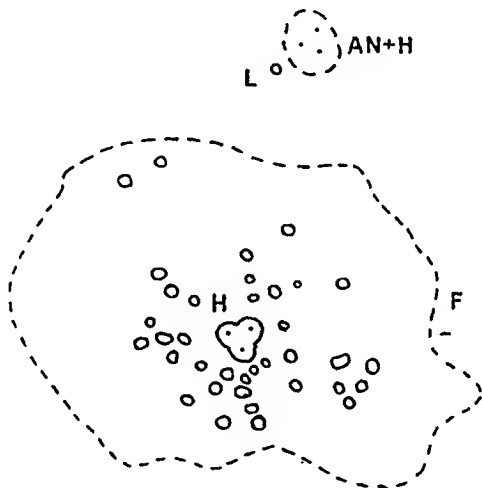


Fig. 5. (Nat. size). Tracing from the forearm into which histamine had been introduced in two areas, the upper one in the diagram anæsthetised. The outline of flare (F) and numerous satellite wheals around the lower histamine punctures are shown, and the area of local anæsthesia which suppressed the upper reaction.

Histamine (1 in 300) was punctured by needle prick into three closely set points upon this, and into three closely set points upon control skin (H). Around the latter a flare (F) soon developed, and satellite wheals began to appear about the 8th minute. By the 14th minute these wheals were full and as outlined. Meanwhile neither flare nor satellite wheal had appeared around the other stimulus. At or about this time however the area of anæsthesia recovered, and by the 25th minute a single wheal (L) had appeared in its immediate neighbourhood. This is one of three similar observations, similar in all respects, even to the appearance of one to three delayed satellite wheals after the anæsthesia disappeared.

Nerve block. A cutaneous nerve to the skin was blocked by 2% novocaine injection, and an area of completely insensitive skin mapped out (Fig. 6 An). A faradic current* known to be of adequate strength was applied to the centre (S) of this insensitive area and continued for 6 min.. Although no trace of current was felt at any time during its passage, the usual reaction of the skin occurred; a bright and widespread flare (F), irregular in outline, came soon and continued. Wheals began at about the 7th minute and were mapped out at the 15th minute (Fig. 6), at which time the anæsthesia was still holding. The correspondence between the area of flare and that enclosing the wheals is strikingly displayed in this tracing.

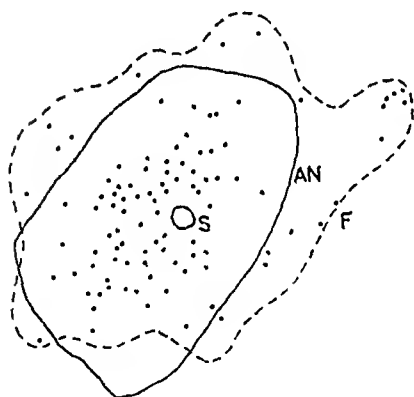


FIG. 6.

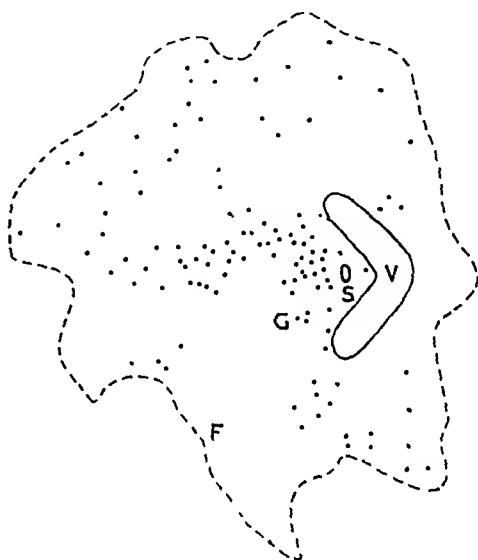


FIG. 7.

Fig. 6. ($\times \frac{2}{3}$). An area of anæsthesia (An) resulting from block of a cutaneous nerve of the forearm. This skin has been stimulated at (S), and a flare (F) has appeared with very numerous wheals, the centres of which are indicated by dots.

Fig. 7. ($\times \frac{2}{3}$). A V-shaped piece of skin of the forearm has been anæsthetised and the skin faradised at S. The area of goose skin (G) of flare (F) and the wheals (dots) are shown in the tracing.

The observation shows that, like the flare, the wheal reaction is independent of intact nerves uniting skin to central nervous system.

Cutaneous barriers. Fig. 7 is one of two similar observations. A V-shaped area of skin was anæsthetised by injecting novocaine intradermally. The faradic current was then applied at S and maintained for 8 minutes at a strength adequate to maintain goose skin throughout. The area of

* This stimulus was used rather than histamine, because on the day this test this reaction was not at its full strength and satollites to histamine were few in number.

goose skin is shown by the dotted line G. A widespread flare (F) soon appeared. Wheals appeared about the 8th minute and were mapped out at the 18th minute, shortly after which time the anæsthetic barrier cleared away. The anæsthetic completely barred the progress of goose skin. The flare, vivid on the stimulus side of the barrier was not completely blocked, appearing on the far side of the barrier though only just distinctly and over a relatively small area. Similarly the satellite wheals were almost confined to the stimulus side of the barrier; and such as appeared beyond the barrier were small and only just distinct. The reactions in this observation, are identical with those which I have previously described with Grant and Marvin (11), so far as goose skin and flare are concerned; they take us farther in showing that the satellite wheal behaves as does the flare.

Pressure with a band. A rubber band 7 mm. wide was passed over the hand and allowed to grip the forearm tightly for a period of $1\frac{1}{2}$ hrs. This device was first used in the hope of obtaining satellite wheals by pressure while the band remained in place, but it gave none. When however the band was removed, the compressed skin at once became very red, and within 15 minutes groups of wheals had appeared upon it and the skin near these was reddened and whealed outside the pressure area for several centimetres. The reason why outside redness and satellite wheals fail to appear during the application of this form of pressure is that it must be applied for such long periods that nerves of the skin are temporarily thrown out of action by the ischæmia. That pressure in band form can block a flare from passing beneath it has been recorded previously (Lewis (9)).

Axonic reflex. The similar behaviour of satellite wheals and flare, their appearance over the same area and in response to the same stimuli, their occurrence in skin to which the cutaneous nerve supply has recently been blocked, their failure when the skin that is stimulated is directly anæsthetised, and the similar influence which anæsthetic barriers exert upon the skin in the two instances, can leave no doubt that flare and satellite wheal are produced through the same nervous mechanism. This mechanism in the case of the flare has been proved to be independent of the sympathetic nervous system, and to depend on nerves of the posterior root system; it is generally acknowledged by workers in this field that the reaction is in the nature of an axon reflex, in an end plexus* of fibres; for, though it still occurs when the nerves are disconnected from the central nervous system, it is abolished as soon as sufficient time has elapsed for these nerves to degenerate.

Cholinergic nerves. In an important investigation published by Grant, Pearson and Comeau (3), cases of urticaria were described in which general whealing occurs in response to heating of a limb. Grant and his collaborators showed that this general whealing is brought about by efferent impulses travelling through cutaneous nerves. It was possible to prevent the

* A plexus, and not a network, as some have erroneously thought us to suppose.

urticarial rash from appearing in a patch of forearm skin by blocking the corresponding cutaneous nerve. And a preliminary rash, a local minute vessel dilatation, would appear on skin and predict the points at which wheals would subsequently come, while the circulation to this limb was blocked, and while therefore the limb was united functionally to the trunk by nerves only. Although they had no opportunity of showing that the general reaction was independent of the sympathetic vasomotor nerves in their patients, there is little reason to doubt that this was so and that it happened through those nerves of the posterior root system which on stimulation give rise to what has been termed antidromic vasodilatation. These authors brought evidence to show that the nerves concerned in the general reaction of their patients are cholinergic. They found that whealing of the skin could be produced readily by passing pilocarpine into the skin electrophoretically, and also by similarly passing a stable acetylcholine compound into the skin, though neither of these procedures would wheal control skins. This demonstration of theirs definitely points to the "antidromic" nerves being concerned, for as we shall see these, according to present evidence, are cholinergic. They were able to exclude sweat nerves as responsible.

That the case here recorded is closely allied to the group investigated by Grant is evidenced by the similar general reaction to heating a limb which all displayed. But to clinch the matter I have used the same tests on my own case to display the involvement of cholinergic nerves.

Pilocarpine nitrate was driven in from the anode (circular and of 22 mm. diam.) of the apparatus described by Wayne (18); the voltage was 8, and the current 21 microamps passed for 10 min.. The surface of the anode was a plug of soft wet cotton wool, and the greatest care was used to avoid pressure on the skin. This produced uniform reddening of the skin but no whealing; the reddening was seen not only where the anode touched the skin but surrounding it where the pilocarpine solution had leaked on to this skin. The observation was repeated, passing 40 microamps for 10 min.; similar reddening occurred and the skin presented 2 small wheals. If saline was substituted and the same amount of current passed, a number of small red speckles were found later on the skin, a delayed and very weak reaction. Pilocarpine solution standing on the skin without passage of current gave no reaction. Pilocarpine was ionised with the stronger current into the arms of three control subjects; just perceptible reddening was seen in two of these, but none in the third, and in no case was there whealing. Briefly, the patient showed a very distinct susceptibility to pilocarpine, used in the manner described.

At a time when the patient had been for several days comparatively free from urticaria, and for some hours had exhibited none, 1/10 grain of pilocarpine nitrate was injected subcutaneously. Within 10 min. a number of small urticarial wheals appeared on limbs and trunk and new ones continued to appear, while the old ones grew or became confluent, until the patient was covered with a very thick and widespread eruption. There was,

however, no perceptible increase of sweating and the patient noticed no watering of her mouth. New wheals appeared till the 75th minute, after which the rash subsided.

Atropine, in the form of 60 minims of belladonna tincture, was used at the end of the patient's stay in hospital. Its use brought the urticaria largely under control, the patient enjoying unusual freedom from the skin affection, which recurred fully when for two days the drug was stopped. While under atropine, efforts to provoke satellite wheals were largely unsuccessful.

Carbachol (carbamylcholine chloride) in 0.1% solution was used in another series of tests; this stable choline derivative was ionised into the forearm with 40 microamps for 10 min.. At the end of that time the whole area of the anodal contact was covered with small wheals; the skin around was unaffected. 10 microamps for 10 minutes gave reddening and a few wheals; 5 microamps for the same period gave reddening only.

Three control subjects were similarly tested with the 40 microamps. All gave very slight reddening, but no wheals.

It is clear that this subject reacted unusually to this choline derivative and to pilocarpine and in the same manner as did the cases of Grant, Pearson and Comeau. Acetylcholine is released by the natural action of nerves of the cholinergic system; pilocarpine is especially stimulative to structures under the control of the same nerves. In our own case and in those of Grant's series therefore there is evidence that the urticaria, when demonstrably produced through nerve action, is produced through nerves of a cholinergic system; for stimulation of such nerves will release acetylcholine and this will act on an apparatus, which the introduction of acetylcholine or of pilocarpine shows to be more than usually responsive.

Identity of "antidromic" and axonic system. It has long been suspected that the nerves concerned in "antidromic" action and those concerned in the axonic reflex of the flare, are the same. This idea was indeed debated and expressed in my book (7) in 1927, but it could not then be regarded as proved. New evidence has come. Dale and Gaddum (2) first suggested (1930) that the vasodilatation induced by stimulation of the distal end of the posterior root may result from peripheral release of acetylcholine; Wybauw (21), a few years later, found evidence that this substance occurs in blood returning from the cat's paw exhibiting this form of vasodilatation, and that the reaction is prolonged by eserine. Grant and his colleagues have demonstrated that vasodilatation, as part of an urticarial eruption, occurs through nerves which they conclude to be cholinergic, and the evidence of the case here recorded brings us to the same belief for nerves subserving the axon reflex and the flare. Here is strong evidence that the nerves involved, whether locally in an axon reflex or as outgoing paths from the central nervous system belong to the same cholinergic system. Another link in the chain of evidence which would bring the nerves serving these two vascular reactions together has been sought in the past but has been

lacking. Marvin and I (13) showed years ago that "antidromic" vasodilatation involves the release of a stable vasodilator substance, or H-substance. Our evidence was followed by that of Ungar (16, 17) and his associates who found similar stimulation to result in increased secretion of hydrochloric acid in the stomach, a well-known effect of introducing histamine into the circulation. To bring the "antidromic" and flare reaction into line, the release of H-substance through the axon reflex should be demonstrated.* Until the present time such evidence as we have possessed has not favoured the idea of such release.

I have drawn attention (10) to the difficulty raised by this missing evidence and have been constrained to admit that if H-substance is released in only the one reaction then the difference would be a fundamental one.

The flare from its early investigation has been regarded as the result of an active arteriolar dilatation, in contrast to the local vascular reaction to injury which certainly includes an active dilatation of the minute vessels. The points discussed have been two; firstly is there direct evidence for the release of a stable vasodilator substance in the flare area; and secondly, is indirect evidence of such release to be found from active dilatation of minute vessels in the same area? The first suggestion of such release was made by Grant, Marvin and myself (11) many years ago. Our investigation was undertaken to ascertain if, when the fading of a flare is held up by circulatory arrest, this delay is due to retention of H-substance at the site of injury or in the surrounding area of the flare itself. We were able to satisfy ourselves that the effect is due to retention at the site of injury. The evidence for this conclusion, as we drew it, and the conclusion itself still stand; but our evidence did not exclude, nor was it intended to exclude, the remote possibility that *in addition* to a main release, a secondary release occurs at the terminations of the axon reflex. Krogh and Rehberg (6), (see also Krogh (5)) became interested in this possibility and concluded in favour of it, using indirect evidence, which they believed showed active dilatation of the minute vessels of the flare area. But they relied upon measurements of capillary pressure by a capsule method, and upon Rehberg and Carrier's ghost flares (14), flares supposedly appearing while the circulation to the skin was stopped. Neither of these evidences proved acceptable on close reinvestigation by Haynal and myself (12). Thus the matter has stood, and it has stood so because the secondary release of H-substance in the flare area is normally inadequate to produce effects that can be detected. Our present case of urticaria has provided a skin in which H-substance is released with exceptional facility; this skin, through the satellite wheals, which appear over the whole area of the flare, has provided unmistakably the evidence that has long been sought. Thus the chief

* To avoid confusion I may anticipate by remarking that to-day's evidence favours the release of acetylcholine by the nerve endings (cholinergic nerve endings) and that this in turn releases H-substance from the cells of the skin.

obstacle to our concluding that the two vascular reactions discussed occur through the medium of one system of nerves has gone.

As a separate conclusion we may say that the system of nerves underlying urticaria in Grant's cases and in that here recorded is one and the same; and since the latter occurs in the nerves underlying the axon reflex, which is proved to be independent of sympathetic nervous system, we may also conclude that the urticarial response in Grant's cases is independent of the sympathetic and belongs, as does the axon reflex, to the posterior root system. This latter conclusion may seem at this stage redundant, but its importance will become evident later.

The abnormal factor. In discussing the mechanism of urticaria in their cases, Grant and his colleagues point out that although they have shown the rash to arise through nervous channels, their observations give no ground for believing that the activities of these nerves are in any way abnormal. The nerves are cholinergic, and cholin derivatives themselves provoke an urticaria in concentrations that give no such reaction in normal subjects. They rightly conclude that the skin of their patients responds abnormally to a normal release of acetylcholine. The same argument applies to the present instance of urticaria provoked through an axon reflex. There is no evidence that the nervous impulse, or the acetylcholine release, exceeds the normal; there is direct evidence of increased susceptibility to choline derivatives. The fault, as in Grant's cases, is in the skin itself. It is important to emphasise that in both instances of urticaria provoked through nerve, a physiological phenomenon, the release of H-substance, ordinarily concealed, is brought to light because, owing to change in the skin, the release is an exaggerated one. In support of this view of exaggerated release in the skin we have the wheals produced as a direct reaction to pressure. The pathogeny may be taken one step farther in enquiring whence comes this increased tendency to respond? If normal serum is injected in small quantity (0.02 c.c.) intradermally, it produces a small reaction that is perhaps a little greater than if normal saline is used. A series of such injections was made in four normal subjects and side by side with them identical quantities of the patient's serum were injected. The reactions from the patient's serum were in every case more prominent and in two subjects were so striking as to be obviously abnormal; wheals 11 or 12 mm. diameter surrounded by widespread and vivid flares resulted. Similarly the patient's skin reacted more severely to her own than to control serum. The potency of this patient's serum in producing wheals is not special to our urticarial patient; it has been shown for other cases, and was reported on by Harris (4). It is attributed to a circulating substance of unknown constitution and origin, which, while rendering the skin abnormally responsive to other forms of stimulation, may perhaps in some circumstances itself determine urticarial eruptions.

It may here be stated that the spontaneous eruption varied in intensity from day to day or week to week, and that these variations were accompanied

by corresponding variations in the case with which satellite wheals were to be induced. Such variations very possibly resulted from changes in the potency of the patient's plasma, but this point was not tested.

Nocifensor or pain nerves.

The original conception that vasodilatation from stimulation of posterior roots is due to antidromic conduction through sensory nerves has usually been accepted. Once escape of current to sympathetic fibres was excluded, as it definitely was, this conclusion seemed to follow inevitably. Firstly, no nerves other than sympathetic and sensory were recognised as forming connections with skin and, secondly, all fibres of the posterior roots were believed to be afferent. It will be perceived that the conception has been formed largely by a process of exclusion. Doubts have arisen from time to time that posterior roots possess a purely afferent function, and recently these have been strengthened by the observations of Barron and Matthews (1), and still more recently by Toennies (15), that there are fibres in the posterior roots that carry efferent impulses provoked reflexly and otherwise. As I have said elsewhere (10) the evidence inclines us to recognise that there are fibres other than sensory in the posterior root system, though admittedly it does not constitute proof. An objection to the belief that the posterior roots contain vasodilator nerves, an objection which has carried weight, has been the uncertainty that "antidromic" vasodilatation is ever provoked as a natural response; it has been possible to regard it as an accidental reaction, occurring only under artificial conditions of stimulation. But this objection has gone once we accept urticarial response in Grant's cases to result from impulses travelling out through the posterior root system, a conclusion already stated; for the reaction, in so far as it involves nerve impulses, is as we have seen a physiological one.

The axon reflex responsible for the flare has also been regarded as taking place in sensory nerve plexuses. With the sympathetic nerves again excluded and the nerves involved traced to the posterior root system, this conclusion also seemed justified; but in this instance it was particularly the pain nerve that became suspect, though for reasons that were not altogether manifest.*

* The matter is discussed at some length by Krogh in the 2nd edition of his book (5). It is with regret that I have found myself so frequently disagreeing with the statements and arguments of this distinguished worker and published by him in this the 2nd edition of his book. It would not I think be of any gain to the subject if I replied to more than a few points at issue, since these are for the most part matters of detail and it could not be done within reasonable compass. But it is necessary that I should give and emphasise the warning that in this book and especially in Chapter VI and X, which here chiefly concern us, a number of statements are attributed to me (and subsequently subjected to destructive criticism) which I cannot accept as representing my published views. These views should be checked from the original sources, before they are quoted. The danger of doing otherwise may be pointed to by an illustration. Krogh credits me with the belief that a number of different reactions (ultraviolet light, reactive hyperæmia) are the result of the release of one and the same H-substance (p. 219, 235 and 238). This or similar statement has been quoted by others, who then proceed to criticise it; yet I made no such statement. On page 138 of my book (7) is the statement "Because, after considering reactions of long latency, I am unable to decide whether injuries to the skin bring their vascular

The attribution of these two vascular reactions to a system of sensory (pain) nerves in the skin, is manifestly hypothetical.

In recent times I have described (8, 9 and 10) a form of local hyperalgesia spreading around a local injury and have brought evidence that this occurs through an axon reflex in cutaneous nerves; likewise I have described how a similar hyperalgesia appears in the territory of a cutaneous nerve stimulated distally. These four reactions, two vascular and two expressed as hyperalgesia, I have discussed together under the term *nocifensor effects*, owing to their very close association. My view as it now stands is that these all take place through a single system of nerves belonging to the posterior root system. That is my main and most positive conclusion.

But I am not prepared to accept a conclusion that these nerves are sensory. No-one recognises more clearly than I do myself that my conception of these nerves as belonging to a separate and special order, is also in the stage of hypothesis. That should I think, be clear from my past writings. My purpose in using a new term has been to emphasise a point of view, which I personally have held to be more in accordance with contemporary knowledge than any other, and particularly to persuade my fellow workers to hesitate before accepting the alternative view, which has been put forward and which I myself at one time countenanced, namely, that the nerves in question are sensory. Committed permanently to this nocifensor hypothesis I naturally am not. My responsibility ends if at a given time I sum up the position as it then seems to stand in the light of contemporary evidence. If I again state the position as I see it in summary form I do so now for the only reason justifying it in an original paper, namely, that I am simultaneously bringing forward new and relevant evidence. For the rest, the hypothesis is serving its purpose and will continue for a time to serve it. Whether, as new evidence comes, it continues to stand or, like most hypotheses, falls, I am content to think that such observations of fact as I have described here and earlier will remain as contributions to knowledge whatever interpretations may come ultimately to be put upon them.

responses solely through the action of a common form of normal metabolite . . . I have allowed the definition of H-substance on p. 105 to embrace more than one substance." The definition may be read on p. 105; I had not committed myself to histamine, or for that matter to any other universal agent.

There are further and more immediately relevant errors. Thus, Krogh (p. 133) credits Marvin and myself with recording the opinion that in the flare we have to do with special fibres which are distinct from those shown to produce H-substance when stimulated antidromically. This statement conveys an erroneous expression of my views and is not justified by anything Marvin and I (13) wrote. In my book of the same year (1927) I actually published a diagram (Fig. 68), representing the same nerve fibre to be involved in the two reactions, discussed their relation and refrained, as I have till now refrained, from coming to a distinct conclusion. Again, Krogh's statement (p. 134) in which I am credited with concluding that no H-substance is produced in the area of the flare does not represent the views as expressed in the paper with Grant and Marvin (11); we were seeking the reason why flares are held up by circulatory arrest and our conclusion was that they are maintained by a release at the site of injury and not by release in the region affected by the flare. Here and elsewhere it is clear that my publications have not received the close reading to which they were entitled.

The initial reason prompting the separation of this system of nerves from those of pain, etc., was that a plexus of axonic branches such as the flare (or local hyperalgesia) requires was difficult to harmonise with the generally accepted view of sensory localisation. There was the additional evidence that asphyxia seemingly paralyses the nerves underlying these local reactions long before pain sense is abolished and that cocaine abolishes pain sense before these local reactions are affected. There was a third and very powerful reason in my mind, which I will now state in a fuller form that would earlier have been possible. It has been shown with a high grade of probability that substances can be released in the skin through nerve stimulation; the mechanism is not a simple one; there appears to be a preliminary release of a choline derivative, which in turn acts upon the cells of the skin and causes a release of H-substance; this second release causes the vascular reactions that follow. Other substances appear to be released similarly and to give rise to local tenderness. The mechanism is not an accidental product of artificial stimulation, it is an important and natural mechanism of defence, called into play by physiological impulses flowing out from the central nervous system, or called into play locally through axon reflexes. The nerves concerned may be regarded reasonably as governing certain activities of skin cells. They belong to the group to which Dale has applied the term cholinergic, mainly a group of efferent autonomic nerves including the vagus, the chorda tympani, and the sweat nerves. We are asked under the older conception to believe that posterior root nerve fibres have not single and precise functions, but that in addition to their sensory function, they possess the power of governing the activities of skin cells and vessels. Thus we are asked to believe that a single set of nerve fibres is uniquely endowed with two functions, the one afferent and the other efferent. This is the full implication of the sensory nerve hypothesis, and its acceptance under that hypothesis should be recognised as necessary to it. Incidentally—and this will perhaps present less difficulty—it will be necessary in accepting this hypothesis to discard the idea that localisation depends upon unit representation in the skin and to accept an alternative explanation that the "local sign" depends upon an overlap of fibres from several distinct units. This possibility has appealed to Weddell (19); it is one that I had not overlooked, having discussed it with Professor Gasser five years ago when my first papers dealing with this subject were being published.

What is the chief objection to the conception of a separate and special system of nerves? It is a consideration to which I have from the first deliberately drawn attention, namely, that we have no direct evidence of the structure and anatomical connections of the nerves, which I suppose to be concerned; under my hypothesis they must be nerves belonging to a hitherto unrecognised system. The chief new work done since my hypothesis was first stated, has been work started by the late Professor Woollard and continued by his colleague Dr. Weddell (19, 20). With this work I was

in close contact in its early phases and through the courtesy of these two workers I had the privilege of seeing preparations and discussing with them the cutaneous nerves so beautifully shown by their new method of staining. They found in the skin superficial nerve plexuses, and it has been their endeavour to sort the nerve fibres out into functional categories. From the plexuses they see fibres ending in various forms of corpuscle regarded as serving touch, warm, and cold sensation. They see varicose fibres leaving the superficial plexus branch repeatedly to end in fine, beaded terminals, below deeper layers of epidermis and in the adventitia of blood vessels. These terminals they regard as pain nerves. By cutting some and working on denervated boundaries they have been able to isolate a few naked terminals, which on appropriate stimulation give evidence of pain. Their conclusion that pain is served by naked fibres is a strong confirmation of the views of previous workers in this field. They state (20) that the "plexiform closely interlocked nerves and endings subserving pain are anatomically similar to the complex and branching system of axons postulated by Lewis as being necessary to explain the phenomena of hyperalgesia, flare," etc.. And say further that "the impossibility of demonstrating histologically several systems of plexiform interlocking fibres, establish that these reactions must take place within the one demonstrable system subserving pain." Their contention may be right, but it would rest on much stronger ground if we could feel assured that while each sensory function has its appropriate and exclusive form of nerve ending, we have at last come to the point of finally recognising these, and can feel further assured that all the nerves of the superficial plexuses end in one or other of these recognisable ways. I do not think we have arrived at this degree of exactitude. I agree that there is a strong case for concluding that pain is served by naked fibrils, and that on the basis of this work pain nerves become of all *sensory* nerves in the skin most suspect of being concerned in the vascular reactions. But because the starting up of pain seems to depend on stimulation of naked nerve fibrils, I am not prepared to accept without further evidence that all fibres in skin (other than those assigned to remaining sensory function) and participating in a plexiform closely interlocked system are pain fibres. I am disinclined to accept it on two grounds. First, for the several reasons previously outlined, and because it requires that pain and vascular reaction should use one and the same fibre, a cholinergic fibre. Second, because the histological specimens show a baffling complexity of interlacing cutaneous nerves. It is quite beyond possibility to trace each and every fibre to its appropriate terminal; it is only possible by closest scrutiny to choose and follow a small fraction of these fibres. With such a complexity of nerves, argument by exclusion appears to me a precarious method.

There is weakness in both hypotheses. On one side anatomical weakness, on the other physiological, and we are not yet in a position to conclude finally for one or the other. But when the whole evidence as it stands is passed in review, then of the two hypotheses I continue to support that

which postulates a separate and special system of nerves ; and I do so with less hesitation, because there is present use in this hypothesis. Its consideration has already reopened enquiry into the meaning of sensory localisation, and in other directions is stimulating new work.

There is a final word to add. Several very interesting physiological reactions in the human skin have been discovered and have been investigated, it is known with a considerable degree of certainty that all occur in the same set of nerves, and the intimate mechanism of the individual reactions is in great measure understood. This basic knowledge rests upon precisely the same foundation of evidence whether we accept the nerve system in question to be sensory, or special and nocifensor.

SUMMARY AND CONCLUSIONS.

1. A case of urticaria, with occasional subcutaneous swellings, is described, in which a general skin eruption could be produced by applying heat to the lower limbs.
2. In addition a constellation of urticarial wheals could be produced at and around the site of a quite local cutaneous stimulus, such as heat, freezing, severe pressure, or faradism ; it could be produced around histamine punctures of the skin.
3. The constellation of wheals fell always within the area exhibiting the vascular "flare," and when sufficiently extensive corresponded closely with the distribution of the latter.
4. As in the case of the "flare," so this reaction was found to be dependent upon the functional integrity of cutaneous nerves, but to be independent of the central nervous system. Flare and satellite wheal are regarded as produced through the same nervous mechanism, an axon reflex.
5. Because general whealing of this patient's skin could be induced by injecting pilocarpine subcutaneously, and local whealing by ionising either pilocarpine or an acetylcholine compound into the skin, and because the spontaneous eruption could be controlled by atropine, the nerves involved are regarded as cholinergic.
6. The occurrence of satellite wheals in the area of flare is evidence of H-substance release through the axon reflex. Such a release links the axon reflex effects with "antidromic" vasodilatation. In view of the work of Grant, Pearson and Comeau, and of the present observations, both these effects are now regarded as produced by one and the same system of cholinergic nerves.

7. The abnormal factor in the patient was in the skin itself, this organ being rendered unusually responsive by some abnormal quality of the patient's blood plasma.

8. Between two hypotheses, that which supposes antidromic impulses and axon reflexes to make use of sensory (pain) nerve channels, and that which supposes them to make use of special (nocifensor) nerve channels belonging to the posterior root system, the balance of evidence appears still to favour the latter.

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MEASUREMENT OF DUCTION MOVEMENTS OF THE EYE.

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THIS paper deals with the measurement of movements of the eyeball in the horizontal and vertical planes. The terms adduction, abduction, elevation and depression of the eye are used in the sense of inwards, outwards, upwards and downwards movement of its anterior pole and it is these movements which have been measured. The range of eye movements in normal subjects and patients with Graves' disease has been examined but in this paper only data from the former group are given in detail. The method used has proved in practice to be simple and reliable and appears to merit wider clinical application.

Apparatus and method.

The apparatus is based upon the simple principle illustrated in Fig. 1. A pair of vertical wire "sights," SS, are mounted on a cursor which travels round the arc of a circle, AA, so that the line joining the sights is always a radius of the circle, namely, the line SS is always normal to the arc. The arc is mounted in front of one of the patient's eyes and adjusted for height so that the centre of the sights is at the same level as the centre of the eye. With the patient looking directly ahead at a small light, the cursor is adjusted until the sights SS are viewed in line with the median sagittal plane, POR, of the eye, passing through the centre of pupil P. This position of the eye and the cursor (hereafter referred to as "the central position") is the "origin" from which all movements and measurements are made. The setting of the central position of the cursor is read on a circular scale calibrated in degrees, by means of a pointer attached to the cursor. Now suppose the eyeball rotates through an angle, ϕ , about its anatomical centre O so that its median sagittal plane now lies in the plane P_1OR_1 . If the centre of rotation of the eye and the centre of the arc are exactly coincident as in Fig. 1 (a), the cursor carrying the sights may be moved round the arc to the position S_1S_1 , until the line of the sights will once more lie in the median sagittal plane of the eye. If the pointer reading on the scale is noted, the difference between this reading and the original reading gives the value of ϕ in degrees.

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† Physician in the department aided by the British Empire Cancer Campaign.

In making readings it is necessary not only to align the sights and centre of the pupil but also to ensure that the plane of the former is normal to the surface of the eyeball. This can be checked directly in the central position but must be estimated for rotated positions of the globe. The observer should aim to bring the plane of the sights into coincidence with the median sagittal plane of the eyeball.

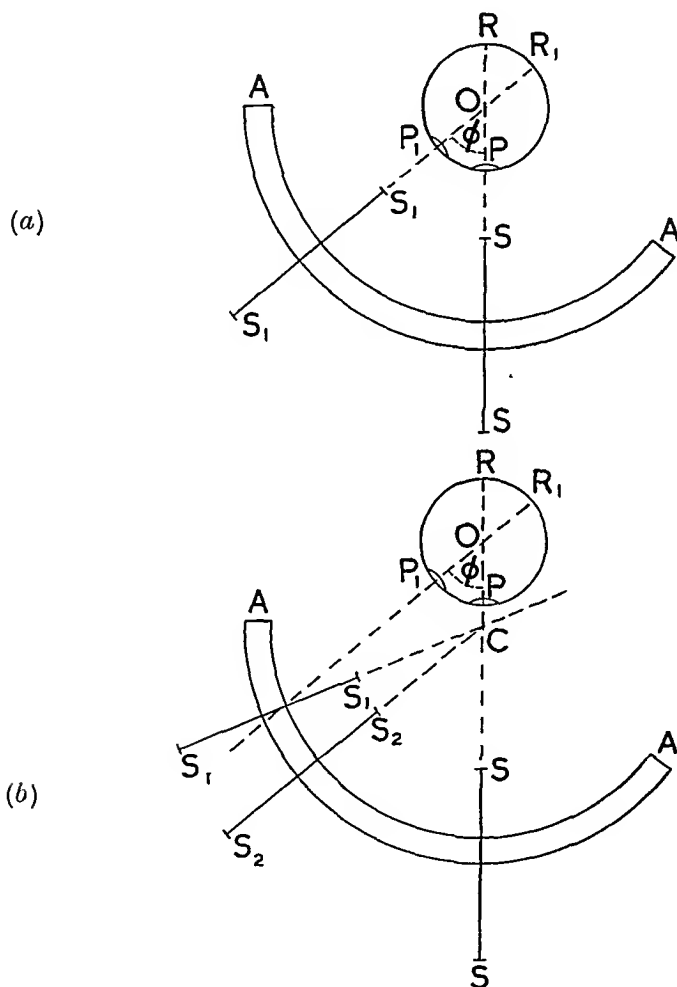


Fig. 1. Diagrams illustrating the principle of the vertometer.

In general, however, the centres of the arc and eye will not be coincident, and when coincidence is lacking it is impossible to arrange for the line of the sights to lie in the median sagittal plane of the eye. Thus Fig. 1 (b) illustrates the case when the centre C of the arc is in front of the centre O of the eye. It is seen how positions of the sights such as S_1S_1 and S_2S_2

cannot bear the required relationship, but the latter position is satisfactory if the arc is moved forward to make the two centres coincident. It is necessary therefore to provide a convenient method whereby coincidence of the two centres may be brought about smoothly and easily. For this purpose the apparatus (arc, cursor and scale) is carried upon a small optical bench along which it can slide easily in either direction. To set the sights in line with the median sagittal plane for a position of the eye such as ϕ therefore requires two adjustments which are made simultaneously; the cursor is moved round the arc at the same time as the latter is adjusted towards or away from the eye.

The description given above is for measurements of adduction or abduction of the eye. It is only necessary, however, to adjust the arc AA in the vertical plane in order to measure elevation and depression; the same principles still apply.

The apparatus, hereafter called the vertometer,* is illustrated in Fig. 2. which shows how the vertometer is carried on the optical bench B and how it may be mounted for measurements in either the horizontal or vertical plane. For horizontal measurements it is used as in Fig. 2 (a), adjustment of the height being obtained by means of either of the clamping screws C_1 and C_2 . For vertical measurements the wooden base W is mounted as shown in Fig. 2 (b) and small adjustments for height are made by means of C_2 . A leaden cylinder L is fitted as shown to serve as a counterpoise. The arc AA (radius of curvature of 15 cm.) of sheet brass 1 mm. thick, is mounted on the wooden base which carries also the graduated scale P. The latter is a sheet of "perspex" with graduations showing intervals of 3° . Since the distance between the graduations is 11 mm. it is easy to read to $\frac{1}{2}^\circ$ and we have taken all readings to the nearest $\frac{1}{2}^\circ$. The cursor which carries the sights S round the arc has a spring fitting which ensures that the line of the sights is always normal to the arc, and also serves to maintain the sights in any required position when the instrument is used for upward and downward measurements. For convenience when measuring movements in the horizontal plane, a second cursor with a pair of sights may be used to determine and keep marked the central position. This fits more loosely on to the arc and can easily be removed. The distance between the sights of both cursors is approximately 12 cm., which is sufficient to minimise errors due to parallax. The sights are of stiff copper wire (22 S.W.G.) and are painted, one red, the other white to facilitate observation.

To maintain the position of the head we have used a chin rest and head clamps which are attached to the table on which the vertometer is mounted. The patient sits on a stool which can be adjusted for height so that any person may be adapted to the apparatus. With the head firmly fixed, strain of the back or neck may be felt during the test unless the stool be

* We are indebted to Mr. N. H. Pierco of the Physics Department for his help with the design and construction of the vertometer.

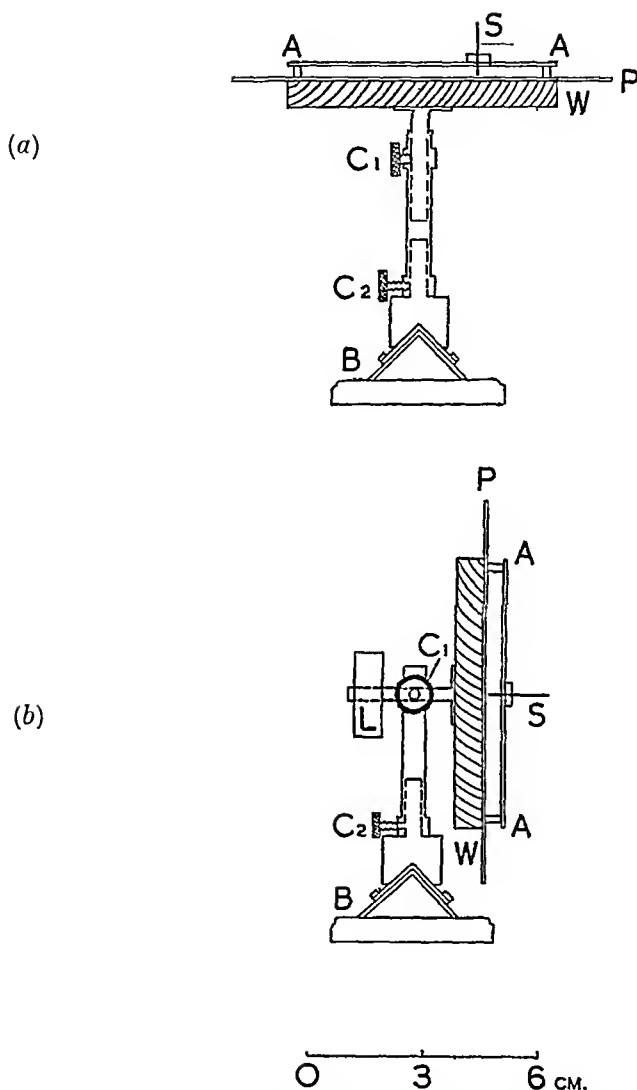


Fig. 2. Vertometer in section (a) adjusted to measure movements of eye in horizontal plane (b) to measure movements in vertical plane.

adjusted accurately for height. For this reason also we have used a stool with an adjustable back. Specifying and maintaining the position of the head and eyes are obviously of great importance. Regarding the head, we keep the plane of Reid's base line horizontal and the median sagittal plane vertical and directly in line with a lamp placed 15' in front of the patient and on the same level as his eyes. We take as the central position of the eyes that in which the patient looks steadily forwards at the lamp. Under these conditions each eye looks directly forwards with an error of less than $\frac{1}{2}^{\circ}$.

The co-linearity of the lamp and A-P plane of the head can be adjusted quite accurately by simple observation, small errors being quite obvious. Reid's base line is set in the horizontal at the beginning and observed continually throughout the test, particularly during measurement of elevation and depression of the eye, to ensure that no movement of the head occurs. This observation is facilitated by means of skin-pencil markings at the lower border of the orbit and on the tragus opposite the middle of the external auditory meatus.

It will be seen from Fig. 1 that setting the vertometer for the central position requires that the optical bench be parallel to the lamp-head line, which is, of course, normal to the eye in the central position. To assist this adjustment the table top carries a series of lines parallel to the required direction. The optical bench is kept parallel to the nearest of these lines. Finally, the sights are adjusted to lie in the median sagittal plane of the eye. This setting is made very accurately by moving the instrument to and fro on the optical bench; it is only correct when the sights remain in that plane in any position on the optical bench. When this condition holds the line of the sights must of course be parallel to the optical bench, and this fact may be used to set the sights in approximately the correct position in advance so that only minor adjustments are then necessary.

For measuring elevation of the eye the vertometer is stood on a small platform 5" high. The same effect could be achieved by providing more adjustment for height in the instrument itself, but this would have led to some instability with our small optical bench.

A number of subsidiary, but important, practical points are as follows:—When any given movement is being measured, the subject is asked to move the eyes *fully* in that direction and to maintain the position while the new setting is made corresponding to the position reached. The reading is repeated a number of times (three or more) and the mean value taken as the range of movement in this direction. The central position is checked after each reading. It is sometimes found that the first reading is somewhat lower than subsequent ones. This occurs when the patient has not grasped the fact that a maximum effort is required of him. The accuracy with which the observer can bring the line of the sights to lie in the median sagittal plane of the eye depends to a great extent upon the adequacy of his view of the eyeball. To ensure an adequate view it is necessary to retract the lids for measuring depression and occasionally also for adduction and abduction. In a few cases pronounced prominence of the supra-orbital ridge or bridge of the nose makes it necessary to estimate the extreme setting in elevation or adduction respectively.* This is never necessary in outward or downward movements. In rare instances the observer's view of elevation may be obscured by bushy or prominent eyebrows*; this difficulty is usually overcome by asking the patient to raise the eyebrows.

* Such difficulties do not arise when these movements are limited in disease. Eyes in which the movements are limited are easiest to examine.

With practice, two observers working together can complete the whole series of measurements (elevation, depression, adduction and abduction of both eyes) in 15 to 20 minutes.

Significance of the measurement. It will be apparent that with the vertometer we measure the angle between the median sagittal plane of the eyeball in the central position and the same plane when the eye is rotated fully in the direction being examined. We have assumed above that the eyeball rotates about its anatomical centre. But the true centre of rotation for adduction and abduction lies somewhat posterior and medial to the anatomical centre (7). In a detailed examination of this point (6, 8, 9) it has been found that not only does the functional centre of the eyeball lie posterior and medial to the anatomical centre, but also that it moves in a small arc with full adduction and abduction of the eyeball. Further, some translatory movement of the globe may occur with extreme abduction or adduction. A brief comment on the relationship of these facts to our measurements of adduction and abduction will be relevant.

We may first consider the effect of the anatomical centre and the centre of rotation not being exactly coincident. Assuming the latter centre to be fixed in relation to both the orbit and anatomical centre, it may be shown that the angle between the median sagittal plane in the central and rotated positions (which we measure) is in fact the same as the angle of rotation described about the true centre.

An examination of the case when the anatomical centre moves relative to the centre of rotation during rotation, shows that even for a relative movement of 1 mm. (which is the probable maximum) the difference between the angle we measure and the angle turned about the centre of rotation is not more than 2° .

Finally, if some slight translatory movement of the eyeball occurs, provided that the rotation and anatomical centres are equally displaced, it may be shown also that the angle we measure is the same as the angle between the lines joining a fixed point on the anterior aspect of the eye (in the central and rotated positions) and the centre of rotation in the central and rotated positions respectively.

It would appear therefore that the measurements made with the vertometer about the anatomical centre of the eyeball are for practical purposes identical with the true rotations in spite of the lack of coincidence of the anatomical and rotation centres and slight movements of the one relative to the other.

Reproducibility of readings (errors of estimation). Possible sources of error in the use of the instrument are (i) inaccuracies in the adjustment of the patient's head; inadequate fixation and maintenance of the head in the correct central position, (ii) errors in setting the sights.

The effect of such sources of error was examined by carrying out a series of measurements on the same subject. All the movements were measured on 11 separate occasions over a period of several weeks. The

TABLE I.

R.C., aged 14. M.

Results of 11 observations.

Observation No.	Right				Left.			
	U	D	I	O	U	D	I	O
1	48	48	45½	53	48	49	49	45½
2	43	51	48	50	44	48½	51	48½
3	44	48	44½	50	46	45½	50	47½
4	45	50	46½	47	44	48	47	48½
5	48	49	51	46	47	50	48	50½
6	46	49½	46½	48	45	49	51½	45
7	48	51½	45½	50½	47	52	48½	45
8	43½	52½	43	49½	42	53	48½	44
9	44½	53½	49½	46	44	54½	49½	48
10	47	49	44½	46½	43½	49	48	47
11	43½	49½	46½	46½	43½	50½	50	46½
Mean . . .	45.5	50.2	46.5	48.5	44.7	50.0	49.2	46.9
Stand. dev. . .	1.98	1.79	2.31	2.30	1.865	2.50	1.365	1.825

Mean standard deviation = 1.99°.

TABLE II.

N.P., aged 35. M. Results of 6 observations.

Mean . . .	39.6	51.2	45.5	48.7	38.8	51.2	48.7	44.1
Stand. dev. . .	1.495	0.700	1.340	1.970	1.365	1.215	1.565	1.88

Mean standard deviation = 1.44°.

B.A., aged 16. M. Results of 6 observations.

Mean . . .	43.9	52.3	43.7	49.3	42.5	53.6	47.4	44.2
Stand. dev. . .	2.27	2.07	1.60	2.07	0.773	1.066	2.15	1.365

Mean standard deviation = 1.76°.

results are collected in Table I where the mean values and standard deviations are also given.† Two other subjects were examined in the same way except that only 6 sets of observations were made in each case. Table II shows the mean values and standard deviations for these two subjects, and in general the results agree with those from the first control. The standard deviations for the different measurements* in all 3 subjects are low and indicate that the method is sufficiently accurate for clinical purposes.

* An additional non-instrumental source of error is of course introduced here for the effort made and movement achieved by the subject on different occasions may well vary.

† In this table elevation, depression, adduction and abduction of the eye are indicated by the letters U, D, I and O. These abbreviations will be used henceforth to represent the movements.

RESULTS.

Control subjects.

Measurements were made of the eye movements in 100 control subjects, 55 females and 45 males, grouped with respect to age as in Table III.

TABLE III.

Age grouping of 100 control subjects.

Age group in years . . .	10-19	20-29	30-39	40-49	50-59	60-69
Number in group . . .	9	22	30	21	13	5

About one-third of the controls were in normal health ; the remaining two-thirds were out-patients with minor diseases. In no case was there a squint or history of major ophthalmic disease. Subjects with high errors of refraction or obvious heterophoria were excluded.

Analysis of whole material. Taking the whole of the material together the usual statistics were calculated for each movement. The results are given in Table IV.

TABLE IV.

Total 100 mixed controls. Summary of statistics.

	RIGHT.				LEFT.			
	U	D	I	O	U	D	I	O
Mean	43.4	50.2	46.2	49.6	42.3	50.0	49.4	47.1
Stand. dev. . . .	2.97	4.10	3.50	5.28	2.90	3.73	4.13	4.28
E (M)	0.297	0.410	0.350	0.528	0.290	0.373	0.413	0.428

From the central position the extreme range of depression of the eye averages 50° ; dextroversion is also 50° and lævoversion slightly less. Elevation is the least free of the eye movements and is regularly associated with a distinct feeling of strain not noticed or complained of with forced depression, abduction or adduction of the eye. The primatial direction of the gaze appears to be slightly downwards with Reid's base line horizontal (2). Hence our average value for elevation may be rather less than is possible from the primatial position. Extreme elevation of the eyes does however involve an unusual muscular effort (1) and the comparatively limited range of movement in this direction and the discomfort it entails may perhaps be correlated with its less frequent use in primates, than other eye movements.

It is of interest to examine the statistical significance of the differences between the mean values obtained for corresponding movements of the right and left eyes. Using the standard P and t test (5) for comparing the mean values, it may be shown that the differences between the mean U and D values for the two eyes are not statistically significant.

The values for dextroversion and lævoversion of the two eyes also show no significant difference.* The data thus provide striking evidence of the perfection of the reflex mechanism maintaining conjugate deviation of the two eyes even in extreme efforts at version. On the other hand the differences between the means for adduction and abduction in the two eyes are more than 99 per cent. significant (P less than 0.01), so that on the average, in normal persons dextroversion is slightly freer than lævoversion. It is difficult to suggest any explanation for this fact although it may be connected in some way with right-handedness.

Effect of sex. The effect of sex may be examined by calculating the same statistics as before, with the material divided into two groups, male and female. The values of these statistics are given in Table V. The statistical significance of the differences of the mean values obtained for identical movements in the two sex groups may again be investigated by the P and t test (5). In no case is the difference in the mean values statistically significant. Table V also indicates that the "right-handedness" previously found in the whole material is present in both sex groups to a significant extent.

TABLE V.

Effect of sex on normal eye movements.

		RIGHT.				LEFT.			
		U	D	I	O	U	D	I	O
Mean . .		44.0	50.6	46.5	49.3	42.8	50.7	48.7	47.7
Males Stand. dev.		2.51	4.60	3.98	5.40	2.30	3.20	4.31	4.06
N=45 E (M) . .		0.375	0.685	0.583	0.806	0.343	0.477	0.644	0.607
Females Mean . .		43.0	49.8	46.1	49.8	42.0	49.3	49.7	46.6
N=55 Stand. dev.		3.38	3.87	3.09	5.20	3.34	3.56	4.09	4.33
E (M) . .		0.456	0.523	0.417	0.702	0.450	0.482	0.551	0.581

Effect of age. To examine the relationship between age and eye movements in normal individuals, the material was divided up into the age groups used in Table III and the mean values for each group were calculated for the U, D, I and O movements of both eyes. In addition the standard error of each mean value was determined. The data thus obtained are represented graphically in Fig. 3.

* Maximum differences between conjugate movements recorded with vertometer were elevation, 8°, depression 7°, lævoversion 11½°, dextroversion 11°. These values fall within the range of instrumental error (cf. Table I).

A separate graph is shown for each of the 8 movements. The mean value for each age group is plotted as a point at the median value of each age group. The height of the line through each point indicates the value of the standard error. The mean values for all the data are shown as a

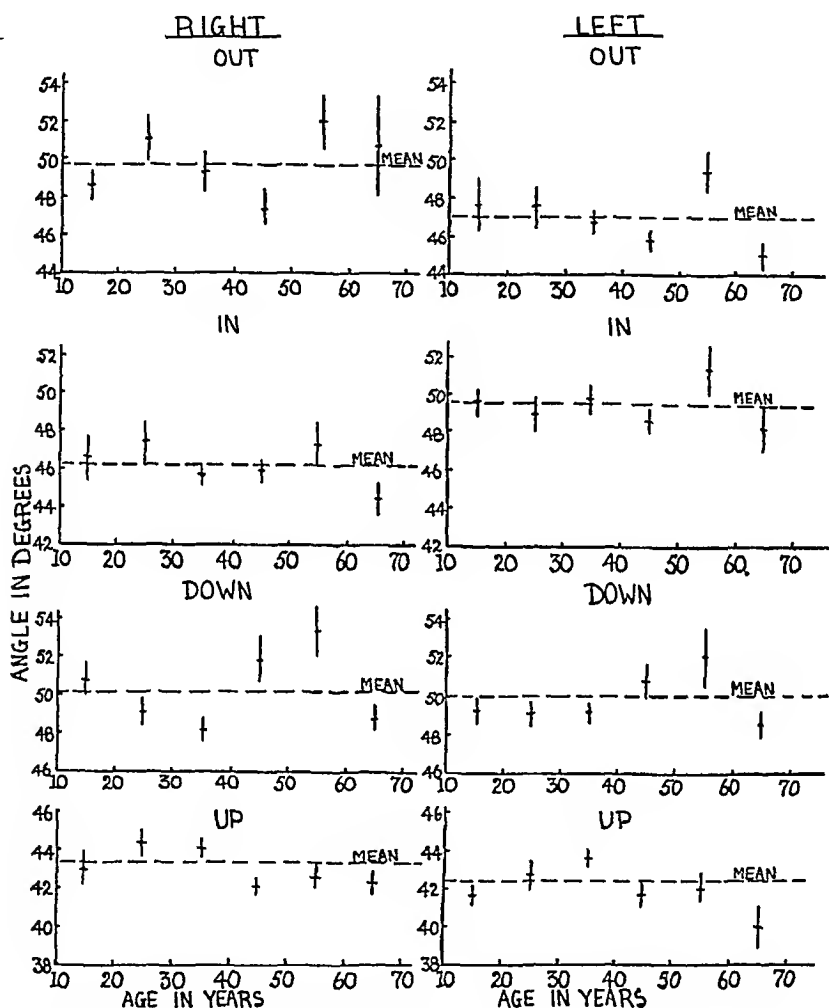


Fig. 3. Range of abduction, adduction, depression and elevation of the right and left eyes in controls of different age-groups.

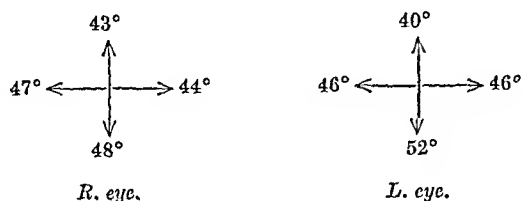
dotted line running across each graph. In all cases the values are approximately balanced about the mean values. The subjects measured were not evenly distributed between the different age groups but the data suggest no clear correlation between age and movement of the eye in any direction.* The graphs also show clearly the magnitude of the differences

* This was confirmed for elevation of the eye by calculating the coefficient of correlation between this movement and age. The value of 0.164 thus obtained is not statistically significant.

between the mean values of the various movements for right and left eyes.

Range of conjugate movement of eyes. By the method described the range of conjugate movement of the eyes, with the head fixed, may be roughly computed; the lesser of the two values for versions upwards, downwards, inwards and outwards may be taken as the corresponding conjugate range. The total conjugate range in the vertical or horizontal plane equals the sum of the conjugate ranges upwards and downwards or inwards and outwards respectively.

For example, suppose that in a given subject the mean ranges of movement in the cardinal directions have been determined as follows:—



The range of conjugate movement upwards may be taken as 40° while the total ranges of conjugate movement* in the vertical and horizontal planes are 88° and 90° respectively.

The vertometer has been used to measure the range of binocular and monocular vision allowed by the visual apertures of various respirators and goggles. For example, the range of inward and outward movement which can be "effective" is given by the reading on the vertometer when the median sagittal plane of the rotating eyeball impinges on the margin of the visual aperture. By comparing the effective range possible in a respirator with the normal the amount of "mask interference" may be determined.

Examples of clinical application.†

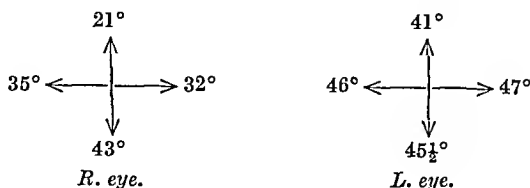
Case 1. M.P., a woman aged 44, suffered from meningioma of R. fronto-temporal region involving posterior part of orbit. Gross proptosis of R. eye, (exophthalmometer reading: R. 28.5 mm, L. 16.5 mm.). Sclera of R. eyeball exposed between limbus and lid margin above ($\frac{1}{2}$ mm.) and below (2 mm.) cornea. R. eye displaced slightly downwards as compared with the left, but optic axis directed normally forwards.

* In subjects where adduction has to be estimated because of the prominence of the nose the true range of conjugate movement may exceed the "effective" range. The effective range of adduction is taken as the angle between the central position and the reading where the median sagittal plane of the inwardly turning eye impinges on the bridge of the nose. From this reduced value the effective range of conjugate movement may be calculated.

† We are indebted to Dr. S. P. Meadows for referring these patients to us for investigation of the eye movements.

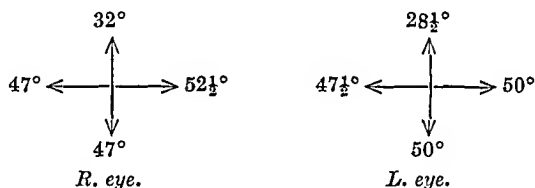
Right pupil sluggish to direct light but consensual reflex brisk. Optic discs normal. Central scotoma, spreading towards periphery on R., L. eye normal.

The eye movements may be shown diagrammatically.



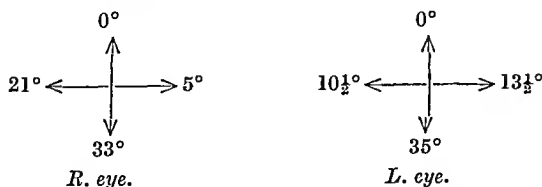
Thus, the movements of left eye are within normal limits. The right eye shows considerable limitation of movement, presumably mechanical in nature and related to the gross mechanical proptosis.

Case 2. V.D., a woman aged 29, presented severe Graves' disease. Marked lid retraction but no clear proptosis (exophthalmometer reading R. 19.5 mm.; L. 18.5 mm.).

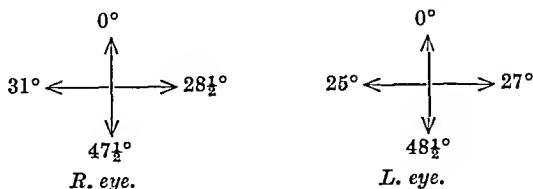


Thus, elevation of both eyes is definitely limited though other movements are full. This finding is common in Graves' disease.

Case 3. G.D., a man aged 56, suffered from myasthenia gravis. The eye movements were measured before prostigmin, while ptosis was present on both sides, though more on the left side.



An intramuscular injection of 1½mgm. of prostigmin was given at 3.25 p.m., and the eye movements remeasured at 3.38-3.55 p.m.



The ptosis was relieved, in fact the upper lid on the right side became retracted.

Thus, all the movements of both eyes apart from elevation show a striking increase after prostigmin. Depression becomes normal. The relative freedom of downward movement before and after prostigmin is in striking contrast with the complete lack of power of elevation.

Interpretation of limitation of ductions.

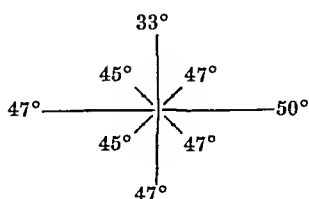
The vertometer is designed essentially to measure movements of the eye in the horizontal and vertical planes. Measurements of abduction and adduction of the eye provide a reasonably satisfactory method of estimating the power of the external and internal recti, for although other muscles co-operate in these movements their motor action is limited and therefore any marked deficiency of abduction or adduction must be attributed to weakness of the corresponding rectus (3). Abduction and adduction therefore rank as diagnostic directions of the gaze but with ductions straight up and down from the central position the case is different since the recti and obliques share the burden of these movements. In testing the vertical motors of the eyeball the oblique directions of the gaze are diagnostic. Thus in moderate abduction the inferior oblique no longer exerts any elevating action whereas the superior rectus functions at its greatest mechanical advantage; therefore, to demonstrate best an isolated paralysis of the superior rectus, elevation must be tested in this position of the eye. On the other hand in full adduction the work of elevating the eye falls on the inferior oblique, the superior rectus having lost, almost entirely, its elevating action. Therefore inability to look upwards and inwards is due to paralysis of the inferior oblique. Since the combined effort of the superior rectus and inferior oblique in elevating the eyeball from the central position is greater than that achieved by either muscle working independently it is important to adjust the eye accurately in the central position before measuring elevation and depression. Deviations of the optic axis during measurements should also be corrected. Limitation of upward movement or uncontrollable deviation of the optic axis from the sagittal plane indicates underaction of one or other of the vertical motors and the necessity for testing movements in the oblique directions of the gaze. Thus measurement of upward movement with the vertometer is of value as a preliminary to the examination of the function of the vertical motors.

Where limitation of eye movement is not due to paralysis of a single muscle, for example in the gross mechanical proptosis of orbital tumours, or in myasthenia gravis, it is clearly best to measure and record movements in the cardinal planes. The advantage of the vertometer is that it reveals insufficiencies of muscular action that are slight and perhaps have caused no restriction of movement or diplopia within the limits of the ordinary field of movement which is small and stated by Duane (3) to be no more than

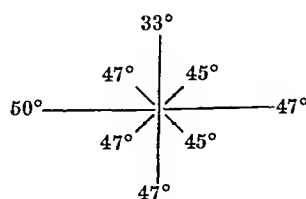
10° — 12° in all directions. Again even greater limitation of movement, if symmetrical, causes no diplopia and may be overlooked unless movements are specially measured. The vertometer provides an objective method of recording limitation of eye movements which is independent of the power of vision. The range of conjugate movement as measured above is not the same as the field of fixation.

Summary of previous methods.

Duke-Elder (4) mentions two objective methods of measuring eye movements, the perimeter and Stevens' tropometer. With the former a small light is made to travel along the arc. The head is kept stationary on the rest but the eye follows it and the angle of maximal movement in a given direction is indicated when the corneal reflex leaves the centre of the pupil. Apart from minor modifications necessary to fix the head during the test and to allow the zero position to be checked easily, it is clear that the perimeter suffers from the fundamental defect that there is no provision for carrying its arc towards or away from the eye so that its centre coincides with the centre of rotation of the eyeball. In prognathous subjects a line joining the ends of its arc may pass 2 inches in front of the corneal apices. Further, the use of a sufficiently strong light to provide a definite corneal reflex may cause epiphora in normal subjects and render the measurement difficult in patients with photophobia or conjunctivitis. But Peter (10) states that he has found the perimeter satisfactory for determining the limits of fixation. Letters in large type are carried outwards along the arc and the point at which the type first becomes blurred marks the extreme angle of fixation. Peter takes the figures for the extreme limits of fixation as corresponding with those of actual rotation which he states are as follows :



R. eye.



L. eye.

Since Peter has really measured a different function there is no point in comparing our data closely with his, but from rough visual estimation it is clear that the eye can turn upwards through about half a right-angle and that his figure for elevation is too low. Moreover, Duane (3) using the perimeter, measured the field of monocular fixation and found the extreme upward range to vary from 40° to 50° . He emphasizes the fact that the angle of fixation does not correspond exactly to, or possess the clinical importance of, the angle of rotation of the eyeball.

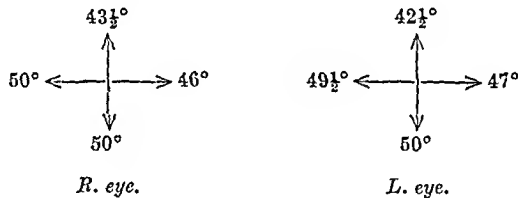
Stevens' tropometer is freely quoted in books of reference as an objective method of measuring eye movements, but his description of this instrument (11) is lacking in essential details and it is therefore difficult to estimate its accuracy. Stevens gives the following values for eye movements as measured by the tropometer :—"Upwards, 33° to 37° ; downwards, 45° to 50° ; inwards, about 50° ; outwards, about or rather less than 50° generally 45° ."

Park (9) has devised an instrument which could be adapted to measure adduction and abduction of the eye. It includes a device for moving the measuring telescope towards or away from the eye so that it lies in the plane of the visual axis. But he has applied it to an investigation of the rotation centre of the eyeball and not the range of duction movements.

SUMMARY.

1. An instrument and method are described to measure the range of abduction, adduction, elevation, and depression of the eyeball. The significance of the measurements obtained is discussed. Errors of estimation by the method are sufficiently small to make satisfactory clinical work.

2. In 100 normal subjects the mean values for these movements may be represented diagrammatically as follows :—



3. No correlation was found between eye movements and age or sex but there was a significant if slight difference between movements to the right and to the left, the former being greater than the latter.

4. The clinical applications of the instrument are indicated and discussed.

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THE ASSAY OF RENIN IN RABBITS WITH EXPERIMENTAL RENAL HYPERTENSION.*

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THE observations recorded in this paper, made between 1937 and 1939, have sought to determine whether any tissues of the rabbit with experimental renal hypertension contain abnormal quantities of the renal pressor substance, renin. It was hoped that the information might help to elucidate the mechanism of renal hypertension and might suggest means of identifying such forms of human hypertension as are of similar origin.

In 1936 Harrison, Blalock and Mason, (11) and Prinzmetal and Friedman (25), working independently with dogs, found that intravenous injections of saline extracts from the ischaemic kidneys of animals with hypertension produced in the unanaesthetised test animal greater rises of arterial pressure than similar extracts of normal kidneys. These observations were the first to suggest that ischaemic kidneys might contain an excess of renin, but the extracts contained depressor substances, producing a preliminary fall followed by a prolonged rise of arterial pressure which did not reach a maximum till 26 to 30 min. after injection, in the experiments of Harrison and his colleagues; renin produces its maximum effect much earlier. Subsequently Harrison and his colleagues (12) repeated the observations testing saline extracts of dog kidneys on anaesthetised rats, and finding greater pressor effects from ischaemic than from normal kidneys; but neither the initial arterial pressure nor the interval between injections were controlled. Recently Landis (15) has freed saline extracts of human kidneys from most of their depressor material by heating to 56° and subsequent filtration, and has tested these on anaesthetised rabbits after nephrectomy. He found no essential difference between control kidneys and kidneys from nephritic and benign hypertension; larger responses were obtained in some cases of malignant hypertension.

The size of the pressor response obtained in a given species from suitable extracts may suffice to detect differences in renin content when these are

* Work done on behalf of the Medical Research Council.

† Research Fellow of the American College of Physicians.

large, but two factors prevent its general use for quantitative work. The first is the variation in the sensitivity of different animals of the same species. The second is the lack of a linear relationship between dose and response as we have shewn in the rabbit; in the dog Swingle, Taylor, Collings and Hays (29) found response proportional to dose over a very small range, but it is unlikely that a similar relationship could hold for larger doses. These and certain other difficulties are avoided in the method here used.

Methods.

Rabbits were used throughout, and were fed on a mixed diet of oats, bran and cabbage. Arterial pressures were measured in the central artery of the flushed ear by Grant and Rothschild's capsule (10). Hypertension was produced, in a manner previously described in detail (24), by constricting one renal artery with a clamp of known size about two weeks after the other kidney had been removed or rendered functionless by ligating its artery or ureter; or by constricting both renal arteries.

The renin content of the kidneys was assayed by the method previously described in detail (23). Extracts of the pulped fresh kidney were made by three methods:—

(1) *The alcohol method.* The pulped kidney was treated with 2 c.c. alcohol per g. for 24 hrs., and the residue separated by filtration through paper and dried at room temperature. For assay the dry powder is extracted with 10 c.c. Ringer per g. for 24 hrs..

(2) *The total globulin method.* A Ringer extract of kidney (4 c.c. per g.) is half saturated with ammonium sulphate, and the precipitate separated by filtration and subsequently dialysed free of sulphate. After adjusting the volume of the contents of the dialyser, the solution is assayed on the day of preparation.

(3) *The heating method,* based on that described by Landis, Montgomery and Sparkman. Pulped kidney is agitated for 1 hr. with 8 c.c. Ringer per g., and the mixture centrifuged. The supernatant fluid is heated to 56° C. for 20 min. and, while still warm, filtered through paper. The resultant solution, kept in the refrigerator, is assayed within 48 hrs. of preparation.

Extracts obtained in this way were assayed by determining the dose which, in a given unanæsthetised rabbit, produced the same rise of arterial pressure as a given dose of a standard preparation of renin, chosen to give a response between 20 and 40 mm. Hg. The standard preparation used here was that described previously (23). It was a dry powder prepared in February, 1937, by extracting rabbits kidneys in bulk with 2 c.c. alcohol per g., drying the residue at room temperature, and after reducing to a fine powder, storing it in sealed tubes at 3° C.. Twenty-four hours before the assay the contents of a tube were weighed out, and extracted overnight

in the refrigerator with 10 c.c. Ringer per g.. During the 2½ years it was used the standard powder showed no more than the slightest loss of activity. Thus in 1937 0.375 c.c. of the standard solution (0.375 unit) produced an average rise of arterial pressure of 26 mm. Hg (30 injections into 11 rabbits). In 1939 the same dose gave an average rise of 24 mm. Hg (44 injections into 16 rabbits).

The renin contents of the kidneys assayed is described in units per g. fresh kidney. By a unit is meant the amount of renin contained in 100 mg. of the standard powder, and thus in 1 c.c. of the standard solution prepared for assay. It is to be emphasised that this unit is not the same as that subsequently adopted by others.

One standard powder no longer exists, but the responses to 0.375 unit already mentioned may help to correlate the data in this paper with that obtained by others. We would emphasise that the response to a given dose by different rabbits is quite variable and that a given rabbit shows some variation from day to day. Thus in 3 months of 1937 0.25 unit gave the following responses (mm. Hg):—

Rabbit 73 : 15, 10, 13, 12.

Rabbit 75 : 15, 17, 19, 21, 13, 11, 20, 19, 16, 18, 21, 20, 18, 21.

Rabbit 77 : 30, 31, 26, 28, 30, 27, 28, 28, 25, 30, 26, 33.

There is thus a considerable probability of error in judging the amount of renin simply from the size of response in the rabbit.

The accuracy and limitations of the method of assay.

Methods for the assay of renin have recently been criticised by Page (18). The criticisms which apply to the method here used are : that the extracts are contaminated with other vaso-active substances ; that the extent to which the methods of extraction recover renin is unknown ; that the responses of the test animal are upset by tachyphylaxis, and that the standard preparation is crude.

These criticisms can be met. The three extracts used have been selected because they are the simplest methods of extraction which yield a pure pressor response in the unanæsthetised rabbit, unaccompanied by any change in the naked-eye appearances of the ear vessels. More elaborate methods of extraction give varying losses of renin at each stage, and the losses are difficult to control. Completeness of recovery of added renin cannot be judged in the case of the alcohol method, because as we have previously pointed out, renin extracted from the kidney is nearly completely destroyed by alcohol at room temperature, although as it exists in kidney it is more resistant (23). Renin in solution can be completely recovered by the globulin method. The heating method, as will be seen later, gives very good yields of renin, but it should be remembered that while renin is apparently not destroyed by the precise procedure named it is destroyed completely by 2 hr. at 60° C..

We have been at pains to point out that tachyphyllaxis does not occur in the method of assay as used by us, in which the animal is unanæsthetised, and in which the doses of renin are small and separated by intervals of an hour or more during which the arterial pressure returns to normal. Tachyphyllaxis does occur in the unanæsthetised rabbit if the dose of renin is very large, and particularly if large doses are repeated at small intervals.

Finally the standard preparation is admittedly crude, but it has had the great advantage of being stable for at least $2\frac{1}{2}$ years ; a similar preparation

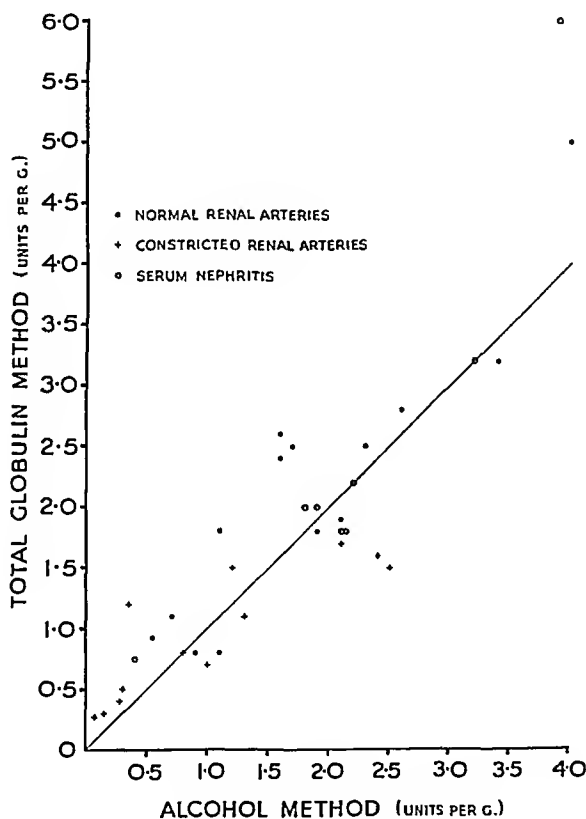


Fig. 1. Shows the relationship between the values obtained for the renin contents of 34 kidneys extracted by both the alcohol and the total globulin methods.

of the posterior pituitary gland has acted as the international standard for many years.

An idea as to the limitations of the methods used may be obtained from Fig. 1, which compares the renin contents of 34 kidneys each extracted by both the alcohol and total globulin method, the extracts being separately assayed. The kidneys were from normal animals, from animals with constricted renal arteries, and from animals with nephritis produced by

Drs. Arnott, Kellar and Matthews by the serum method of Masugi. It may be seen that while the points rarely fall on the straight line indicating equality they are grouped around it. There is no evidence of a systematic error restricting the points to one side or the other, although on an average the yields by the total globulin method tend to be rather higher than those obtained by the alcohol method of extraction. The scatter of the points about the line of identity exaggerates the error of the method, since it is compounded of the errors in the two separate extractions and assays.

It is evident then that while the method of assay is of no great precision, it is well able to detect the considerable variations that occur in the renin

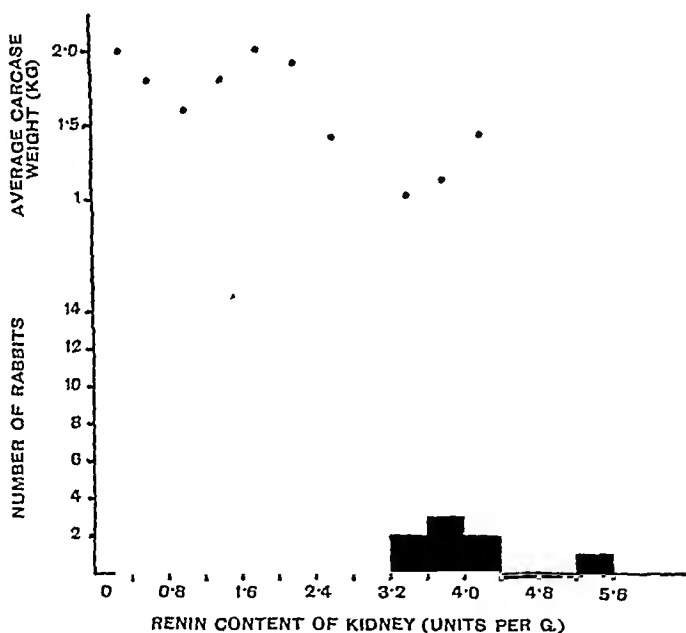


Fig. 2. The frequency and distribution of the renin contents of the kidneys in 60 normal rabbits. The extracts were prepared by the alcohol method. The average carcass weight in the various groups is shown above.

content of the kidneys, for when a low yield is obtained by one method it is obtained by the other, and a similar statement applies throughout the observed range.

While both the total globulin and alcohol methods have been used in each of the groups of animals that follow, the alcohol method has been used more often because of its convenience in giving at one stage a stable product. Unless otherwise stated the renin contents of kidneys subsequently mentioned have been obtained by this method.

The renin content of normal rabbits' kidneys.

As our work proceeded, it became clear that the renin content of rabbits' kidneys showed a much wider variation than we had originally anticipated. Fig. 2 summarising the results obtained in 60 normal rabbits indicates the range and frequency of the variation and the relationship to carcase weight. While nearly half the animals had renin contents lying between 0.4 and 1.2 units per g. kidney the range of variation is between 0.2 and 5.6 units per g.. It will be seen that when the renin contents is less than 2.4 units per g. kidney the average carcase weight is over 1.5 kg. while with greater renin contents, the carcase weight is lower.

It is clear that with so large a variation among normal kidneys, any difference which might be shewn by the kidneys of animals with hypertension would have to be of considerable magnitude to be judged significant. It seemed too that any clue we could get concerning the cause of this variation in normal animals might have an important bearing on the function of renin. We therefore tried to discover whether the renin content of the kidneys was related to any factor within our control. We investigated the method of killing, the food and water intake for the few days before death, external temperature during the same period, sex, and weight. We must emphasize that our experiments have not been conducted on any very large scale. Our objects were twofold: Firstly to determine whether in small groups of animals in which these factors had been as nearly standardised as possible the kidneys still showed wide variations in their renin content; this we found to be the case. Secondly to determine whether the renin content of such groups as were comparable showed any difference suggestive enough to make it worth planning an experiment on a larger scale; they showed no such difference. Since the results of this part of our work are entirely negative, no useful purpose would be served by tabulating them in detail. We will therefore simply record that we have found no significant difference in the renin content of the kidneys of rabbits killed by a blow on the head, by nembutal or chloroform injected intravenously, by bleeding under nembutal or ether anaesthesia or bleeding after a blow on the head; that we have found no significant difference in animals fed on cabbage alone, oats alone, or on a full mixed diet for periods up to six days before death, or on water alone for two days before death; that the average renin content of kidneys has shewn no tendency to change with the season of the year, and that we find no constant relationship between the renin content of the kidneys and the sex of the animal. The influence of age has not been investigated, but that it may prove to be an important factor is suggested by the tendency for high renin contents to be associated with low carcase weights as Fig. 2 shows; and in the only five rabbits with carcase weights under 1.2 kg. (but over 1.0 kg.) the renin contents of the kidneys were 2.6, 3.4, 3.5, 3.8 and 3.9 units per g. values which are well above the average normal.

TABLE I.

The renin content of the kidneys of rabbits with chronic hypertension.

Rabbit	Carcase weight g.	Arterial pressure mm. Hg		Duration hyper-tension	Arterial lesions	Kidney		Renin content kidney Units per g.		
		initial	final			side	weight g.	alcohol	globulin	heating
A. Animals with both renal arteries constricted.										
A. 54	1650	70	135	2 mo	++	L R	12.5 7.5	0.9	0.7 0.2	
138	2350	77	125	4 mo	+	R L	11.2 3.2	0.1		0.2 16
22	2000	80	125	17 mo	0	R L	8.9 2.2	0.1		0.2 2.5
116	2350	75	130	5½ mo	+	R L	12.2 3.8	0.3 0.2	0.5	
115	1060	66	100	3 mo	0	R L	10.1 3.6	40 0.3		
126		86	105	5 mo	0	L R	9.1 1.4	1.0 1.1		2.7
B. Animals with one kidney, the left renal artery constricted. Right kidney removed.										
39	1550	82	145	4 mo	++	L	11.0	1.2	1.5	
46	2100	92	129	5 mo		L	15.3	2.0		
41	1350	74	100	6 mo	0	L	7.5	2.4	1.6	
49	2500	80	96	6 mo		L	11.2	0.8	0.8	
Right renal artery ligatured.										
38	2120	66	115	5 mo	+	L	13.1	1.3	1.1	
47	2000	74	104	5 mo		L	11.4	1.2		
41	1950	78	105	5½ mo	0	L	8.9	2.5	1.5	
Right ureter ligatured.										
48	1600	77	135	2 mo	++	L	9.8	1.6		
43	2000	77	129	4½ mo		L	8.6		0.9	
C. Control animals with one functional kidney, the renal artery not constricted. Right kidney removed.										
61	2300	75	67	5 mo		L	9.3		1.8	
63	1250	79	74	5 mo		L	9.8		0.8	
Renal artery ligatured.										
64	2200	80	85	5 mo		L	11.6	0.7	1.1	
62	2200	72	76	5 mo		R	9.3	0.9	0.8	
Right ureter ligatured.										
68	1800	87	82	3½ mo		L	8.0	0.5	0.6	

The renin content of the kidneys in animals with hypertension.

Chronic hypertension. Table I shows the renin content of the kidneys obtained from 15 rabbits in which the renal arteries had been constricted by clamps with an internal diameter of 0.5 to 0.7 mm. (24) and in which the arterial pressure had been raised for 2 to 17 months before the animal died or was killed. In 6 animals both renal arteries were so constricted; in each of these after death one kidney was found to be atrophied and the other hypertrophied; these kidneys were separately extracted and assayed. It will be seen that of the 12 kidneys, 7 gave values between 0.1 and 1.0 units per g., i.e., were below or within the lower normal range; while only two gave abnormally high values, the larger kidney of rabbit 115 having the extremely high value of 40 units per g. by the alcohol method and the smaller kidney of rabbit 138 having 16 units per g. by the heating method.

In 9 animals the right kidney was removed or destroyed by ligature of its artery or ureter two weeks before the left renal artery was constricted. What remained of the right kidney at the time of death was extracted and tested but in each case the extracts were inactive. The renin content of the enlarged left kidney is shewn in the table. It will be seen that in each case the renin content was well within the normal range. For comparison the renin content of the intact kidney is shewn in 5 animals in which the other kidney had been removed or destroyed by arterial or ureteric ligature $3\frac{1}{2}$ to 5 months before the animal was killed. These animals were precisely comparable to those with hypertension except that the remaining kidney had not been disturbed and there was no hypertension; the range of their renin contents was similar.

In the assay of the kidneys, extracts were prepared by all three methods, because it seemed possible that some pressor substance might occur in the ischæmic kidney with chemical properties other than these of renin. Inspection of the table shows that while the results obtained by the several methods were rarely identical, yet, where in a given kidney, a low or high yield was obtained by one method a low or high yield was obtained by the other. The heating method gave higher yields than the alcohol method in the three instances where both methods were employed and may prove to give the best recovery of renin from the kidney.

Acute hypertension. A major difficulty in recognising any changes that may occur in the renin content of kidneys as a result of diminishing their arterial supply is the great variation in normal animals. It seemed possible that this difficulty might be avoided by comparing the renin contents of the two kidneys of a given animal, the right kidney being removed at a first operation to serve as a control, the left renal artery being constricted two weeks later and the kidney obtained when hypertension had developed. It is necessary first to inspect the results (Table II) of a control procedure in which the left renal artery was exposed but not clamped two weeks after the right nephrectomy. Nine rabbits were used and the left kidney was

TABLE II.

Compares the renin contents (alcohol method) of the two kidneys, the right being removed by nephrectomy two weeks before exposing but not clamping the left renal artery. No hypertension.

Rabbit	Days since control operat'n	L kidney how obtained	Carease weight kg.	Arterial pressure mm. Hg		Kidney wt. g.		Renin content kidney. Units per g.		
				initial	final	R	L	R	L	L-R
54	0	Nephrectomy	2.1	65	70	6.5	7.9	2.0	2.6	0.6
82	1	Killed	1.9	82	80	7.9	10.2	0.8	2.5	1.7
243	4	Nephrectomy	1.6	74	76	6.4	7.7	1.3	3.2	1.9
247	4	Nephrectomy	1.2	72	76	5.6	7.8	2.6	3.6	1.0
223	7	Nephrectomy	2.1			7.2	8.8	0.9	0.9	0
224	7	Nephrectomy	1.6			5.6	6.6	1.1	1.1	0
225	7	Nephrectomy	1.8			7.3	8.8	1.2	1.1	-0.1
231	7	Nephrectomy		86	92	6.2	8.5	1.1	1.3	0.2
233	7	Nephrectomy		91	92	8.3	10.9	1.4	1.5	0.1

TABLE III.

Shows the renin contents (alcohol method) of the two kidneys, the right kidney being removed by nephrectomy 2 weeks before the left renal artery was clamped. All animals with hypertension.

Rabbit	Carcase wt. kg.	Days since L renal a. constr'd	L. kidney how obtained	Arterial pressure mm. Hg			Kidney weight g.		Renin content kidney. Units per g.		
				initial	highest	final	R	L	R	L	L-R
<i>Rabbits in which all the left kidney was infarcted</i>											
45.2		1	Killed	71	86	86		10.7		0.15	
120	1.75	1	Killed	63	77	77		13.2		0.07	
121	1.75	1	Killed	74	81	49		9.9		0.13	
52A	2.2	2	Killed	80	97	89		13.1		0.5	
81	1.9	2	Killed	84	98	63		15.5		0.13	
47.2	1.8	2	Killed	82	114	80		10.6		0.28	
45A	2.2	2	Killed	75	93	57	10.5	15.1	0.8	0.4	-0.4
100	1.9	2	Killed	78	92	90	8.7	12.5	1.0	0.15	-0.85
<i>Rabbits in which one-third to one-half of the left kidney was infarcted</i>											
189	—	2	Died	84	106	99	6.3	8.0	0.8	1.1	0.3
98		4	Died	80	90	90	8.6	12.5	0.4	2.4	2.0
50	2.1	1	Killed	65	92	89	7.0	10.1	0.9	2.1	1.2
<i>Animals in which none of the left kidney was infarcted.</i>											
84	2.0	2	Killed	76	99	98	8.2	11.0	0.9	3.8	2.9
244	1.3	4	Nephrectomy	32	120	120	5.4	7.9	2.0	3.5	1.5
246	1.4	4	Nephrectomy	81	120	120	6.9	7.2	1.8	4.8	3.0
200	1.6	6	Nephrectomy	81	96	96	7.3	11.3	0.6	1.3	0.7
201	1.6	6	Nephrectomy	80	98	92	5.4	8.0	1.1	3.4	2.3
232		7	Nephrectomy	82	113	110	6.7	7.8	2.3	7.4	5.1
234	2.7	7	Nephrectomy	80	96	96	9.0	12.1	2.1	4.3	2.2
185	1.9	8	Nephrectomy	89	126	123	6.3	11.1	2.0	2.5	0.5

obtained up to 7 days after the control operation. It may be seen that the renin content of the two kidneys is in general similar, differing by 0.6 units per g. or less in six animals, and by less than 2 units in the remaining three, but that what difference there is, tends to lie in an increased renin content of the second kidney.

Table III shows the results obtained in 19 rabbits in which hypertension had been produced by constricting the left renal artery very severely by clamps of internal diameter from 0.35 to 0.5 mm.; the right kidney had been removed 14 days previously. The results are divided into three sections, because the course of the hypertension and the renin content of the second kidney was found to depend on whether or not the left kidney showed necrosis. The first section shows 8 animals in which the internal diameter of the clamp lay between 0.35 and 0.4 mm., and in which the kidney showed widespread areas of hæmorrhage and necrosis when the animal was killed one or two days later. In these animals methylene blue injected into the artery proximal to the clamp did not pass it, but flowed freely from the distal end of the artery when the clamp was removed. As we have previously mentioned (24), such animals show a rise in arterial pressure within a few hours of constricting the renal artery, but this is not sustained and falls to or below normal 24-72 hours after the operation, the animal dying soon afterwards; in 4 of the 9 animals shown this fall had already occurred. In all these animals the renin contents of the kidneys were very low, all being below 0.5 units per g., and 5 below 0.2, the lowest normal value we have observed. In the only two rabbits in which we had previously assayed the right kidney, there was a fall, profound in one animal, in renin content. It seems therefore that when hypertension has been produced by a constriction of the renal artery severe enough to produce necrosis of the kidney, renin disappears from that kidney.

In 3 animals, of which two died, the left kidney was partly necrosed; the remainder appeared normal. These animals seemed to maintain their hypertension, and in each the left kidney contained more renin than the right.

In 8 animals the left kidney, obtained by nephrectomy in 7, was enlarged but otherwise of normal appearance. All of these animals had a significant rise of arterial pressure which was maintained, the final reading being 12 to 39 mm. Hg above the preoperative level; and in all the renin content of the left kidney was greater than that of the right, the increase being 1.5 to 5.1 units in 7 animals. Judged by themselves the renin contents of these 8 ischæmic kidneys are all above the most frequent normal values, and one (232) is above the upper normal limit. The increases in renin content of the left kidney as compared with the right are considerably greater than those seen in control animals. These results therefore strongly suggest that when hypertension is produced by a severe constriction of the renal artery which is not followed by renal necrosis, the renin content of the kidneys is increased, at least during the first 7 days.

The effects of the degree of constricting the renal artery and the duration of the hypertension are shewn graphically in Fig. 3 which shows the renin contents of the kidneys of animals with one kidney. The control animals comprise those in Table I and Table II in which one kidney had been removed or destroyed, either 2-3 weeks or $3\frac{1}{2}$ to 5 months previously, and in which the remaining renal artery was unconstricted and no hypertension developed; these renin contents are distributed in a similar pattern and over essentially the same range as are the renin contents of normal animals (Fig. 2). The renin contents of animals with constriction of the renal artery severe enough to produce renal necrosis, and in which the hypertension is not maintained, are all below the control values. Animals in which the renal artery was constricted severely enough to produce hypertension within 12 hours, but in which the kidney did not necrose and the hypertension was maintained, show, 2-7 days after operation, a renal renin content in the upper normal range or above it. In animals with hypertension of 2-6 months duration the renin content of the kidney is normal.

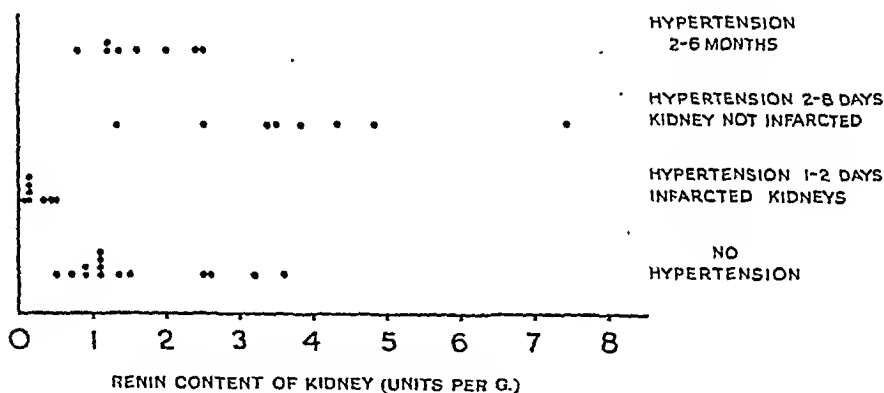


Fig. 3. The distribution of the renin contents of the kidneys (alcohol method) in animals with one kidney.

A search for renin in the blood of the general circulation.

To see whether renin was present in the circulating blood of animals with hypertension, we sought a method whereby the whole of the serum obtained by bleeding an animal might be concentrated, without loss of any renin it might contain, to a volume small enough to inject as a single dose into another rabbit. The following method was finally adopted, aseptic precautions being observed throughout, to prevent the toxic effects of bacterial action on the extracts.

The animal is bled from a carotid artery under ether anæsthesia, the blood being defibrinated by stirring with a glass rod. The corpuscles are centrifuged off and the serum is half-saturated with ammonium sulphate. After standing overnight in the ice-chest, the mixture is filtered and the

precipitate is rubbed up with a little distilled water and transferred to a cellophane bag. It is then dialysed against fast-running tap water for seven hours, after which it is transferred to a Petri dish and dried overnight in a vacuum dessicator over CaCl_2 or P_2O_5 . The dry residue is weighed, powdered and dissolved in 2-4 c.c. of Ringer's fluid. The resulting viscous solution is injected into the ear-vein of an unanæsthetised rabbit, the blood-pressure being measured at half-minute intervals by Grant and Rothschild's method.

Such extracts, prepared from the serum of normal rabbits, usually, but not always, cause a transient rise of blood-pressure, reaching its peak within half a minute after injection and nearly or completely disappearing within two minutes; this can easily be distinguished from a renin effect, which reaches its peak after approximately two minutes.

Recovery of renin from blood when added in vitro. If renin is added to rabbit's blood in vitro and the resulting mixture treated by the method described, the extract gives a typical renin effect in the test rabbit. Because extracts of normal serum are often pressor, it is difficult to say precisely how much renin is recoverable, but probably this amounts to about $\frac{2}{3}$ of that added. For example 80 c.c. serum obtained by bleeding 4 rabbits was divided into 2 equal parts A and B; to B 1 c.c. of a renin solution was added. A and B were extracted as described and the dry residues separately dissolved in 3 c.c. Ringer. 2 c.c. of extract B raised the test rabbit's pressure from 64 to 98 at 1 min., 100 at 3 min., 91 at 5 min. after injection. 40 min. later 2 c.c. extract A failed to raise the same rabbit's pressure by more than 3 mm. above the initial level of 73. 5 min. later 0.6 c.c. of the renin solution originally used raised the pressure of the test rabbit from 70 to 108 at 1 min., 111 at 2 min., and 106 at 3 min. after injection.

Recovery of renin after intravenous injection. In 3 rabbits enough renin was injected to raise the blood pressure by 60-70 mm. Hg and 10 min. later the animal was anæsthetised with ether and bled, and the serum (30, 44 and 61 c.c. respectively) extracted as described; all these extracts injected into normal rabbits produced a pressor response identical with that of renin (Fig. 4).

In 2 other rabbits similar doses of renin were injected intravenously, and 55 to 75 min. later, when the arterial pressure had returned to normal, the animal was anæsthetised with ether and bled and the serum (17 and 42 c.c.) extracted. No renin could be detected in these serum extracts. In both these animals, liver, gut, lungs and spleen were removed after death, and extracted, in one case by the alcohol method, in the other by the total globulin method. With the exception of the liver none of these extracts gave a renin-like response when injected intravenously into rabbits; in both instances the liver extract produced a rise of 20 mm. Hg or more, which was still evident 10 min. after injection. The urine secreted by these rabbits was extracted by the method described in the next section and tested in normal rabbits; no renin was detected in the extracts. In one experiment

the left kidney was removed from a rabbit before, and the right after the injection of approximately 15 units of renin in 4 doses over a period of $1\frac{1}{2}$ hours;* the left kidney (5.8g) contained 1.3 units renin per g. the right (6.9g) 0.7 units per g.. Finally when a mixture of renin and defibrinated rabbit's blood, in proportions similar to that effected by the intravenous injections described, is incubated at 37°C . for $1\frac{1}{2}$ hours, renin can be recovered from the serum by the method of extraction described.

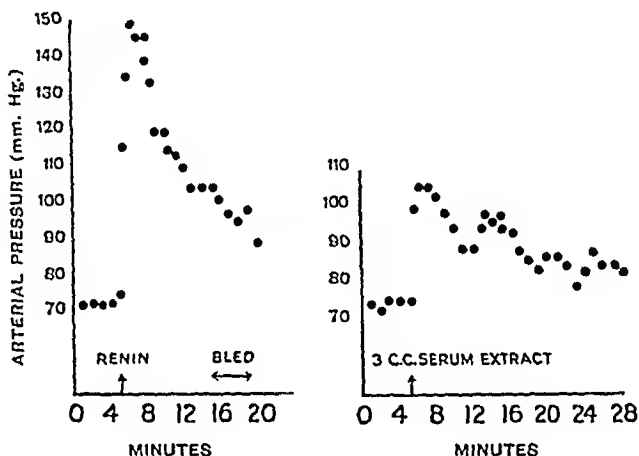


Fig. 4. To show the recovery of renin from blood 10-14 min. after its intravenous injection. The chart on the left shows the response on 1.3.38 of the donor rabbit (2.0 kg.) to 2 c.c. renin injected at the 5th minute. 55 min. later the injection was repeated, the animal was anaesthetised with ether, and bled out from the carotid artery between the 10th and 14th minutes after injection. 30 c.c. of serum were obtained and extracted in the manner described in the text. The chart on the right shows the effect of the extract (3 c.c.) on the blood pressure of an unanaesthetised rabbit (9.3.38).

It seems then that when a large amount of renin is injected intravenously into a rabbit, it is present in the circulating blood 10 min. later, when the arterial pressure is still raised, but has disappeared after one hour when the arterial pressure is normal. We have been unable to recover the lost renin from the urine or the tissues named, nor have we been able to detect its destruction by blood *in vitro*. The fate of renin thus remains undetermined, but it would be a simple matter to take the analysis farther than we have done.

Renin in the blood of rabbits with chronic hypertension. Table IV shows the results obtained by injecting extracts of the blood of 6 rabbits with hypertension of 2 to 17 months duration into normal unanaesthetised rabbits, and the effects of 7 similar extracts of blood from normal rabbits. It will be seen that the extracts of the blood from the rabbits with hypertension

* With such doses and times, the rabbit showed tachyphylaxis.

gave nothing resembling a renin effect, and that the transient pressor action of the extracts obtained from normal rabbits and rabbits with hypertension showed no significant difference in intensity or duration. In 1 rabbit with hypertension of 4½ months duration, we also extracted spleen, liver, gut, and brain by the total globulin method. No evidence of a renin-like response was obtained from these extracts. Prinzmetal, Friedman and Oppenheimer (26) have already reported the absence of any pressor effect of extracts made by this method from the serum of dogs with renal hypertension and patients with essential hypertension.

TABLE IV.

Pressor effects of extracts of serum from rabbits with normal and raised arterial pressures.

Animal	Final arterial pressure	Duration hypertension months	Volume in c.c.		Effect on arterial pressure test rabbit	
			serum	extract	maximum rise	change at 2 min.
54	135	2	55	4	13	—5
43	129	4½	40	1.7	32	+3
22	125	17	40	3	14	+5
138	125	4	50	4	31	—1
116	130	5½	48	3	18	—2
126	105	5	33	3	16	+3
Normal rabbits			36	1.8	12	+6
			60	2	20	0
			60	2	27	+11
			60	2	10	+5
			38	3	19	+5
			30	3	41	+16
			100	3	15	+5

Vaso-active substances in the urine of normal rabbits and rabbits with hypertension.

The question as to whether renin is excreted in the urine is made difficult by the presence of a powerfully depressor substance, kallikrein (7), which resembles renin closely in its physical and chemical properties, and we have been unable to devise a method for separating these two substances. The method we finally adopted and the results obtained in normal rabbits and rabbits with hypertension are illustrated in the following protocol.

Urine was collected for 24 hours from 2 normal rabbits and 2 rabbits with hypertension, kept in metabolism cages and fed on cabbage and oats. The urine from each animal was half saturated with ammonium sulphate. After standing overnight in the ice-chest, the mixture was filtered and the precipitate dissolved in a little distilled water and transferred to a cellophane bag and dialysed free of sulphate. The final solutions were all made up to 19 c.c., and 1 c.c. of each was injected intravenously into 2 normal rabbits with the following effects on the arterial pressure :—

TABLE V.

Urine from :—		Change of artificial pressure in :—			
		<i>Rabbit A.</i>		<i>Rabbit B.</i>	
		Fall	Rise	Fall	Rise
Hypertension	1	17	3	26	8
Hypertension	2	17	8	20	7
Normal	1	20	5	22	3
Normal	2	20	7	32	0

As this protocol illustrates, we have been unable to find any difference between the vaso-active properties of urine excreted by normal and hypertensive rabbits extracted in this way. But it is to be pointed out that the deliberate addition of known amounts of renin to rabbits' urine before testing it by this method has shewn that while large amounts of renin can be recognised, the effect of small amounts may be completely masked by the depressor action of kallikrein.

DISCUSSION.

The hypothesis that hypertension following renal artery constriction is due to the release of renin from the kidney into the blood traversing it has won increasing support, and the evidence has recently been reviewed by Page (22) and by Braun-Menendez and Houssay (3). The chief evidence comes from the dog. In this animal it was first shewn that the hypertension is independent of the nervous connections of the kidney (1), subsequently that the ischæmic kidney raised the pressure of a nephrectomised recipient when grafted into its neck (6), and finally that considerable amounts of renin are detectable in the venous blood leaving the ischæmic kidney (3). Page and his colleagues and the Buenos Aires group have shewn independently that renin does not act directly on the vessels, but liberates from a thermolabile constituent of the plasma globulin fraction (renin activator or hypertensinogen) another substance angiotonin or hypertensin, which

constricts the small arteries and arterioles and which differs from renin in being diffusible, stable to boiling, and alcohol soluble; in this reaction renin behaves like an enzyme (2). Page and his colleagues have provided evidence suggesting that angiotoxin is present in abnormal quantities in the circulating blood of dogs with renal hypertension (19), and also that in such animals the plasma content of renin activator is increased (14).

According to dell'Oro and Braun-Menendez (3) no renin is secreted by the normal kidney transplanted under the skin but evidence for the discharge of a vaso-constrictor substance from normal kidneys under experimental conditions has been obtained by Verney and Vogt (32) and by Govaerts (9).

It is generally assumed that the stimulus to the release of renin from the kidney is a reduction in its blood flow, and experiments by Levy, Light and Blalock (17) support this conclusion. Concoran and Page (5), on the contrary, estimating the diodrast clearance rate in dogs conclude that in hypertension due to perinephritis or to renal artery constriction the renal blood flow is not necessarily reduced. That reduced renal blood flow may release renin into the circulation has been shewn by Prinzmetal, Lewis and Leo (27) who, following Taquinis' (30) demonstration that in the dog hypertension follows the re-establishment of the circulation to a completely ischæmic kidney, have provided convincing evidence for the substance released from the ischæmic kidney being indeed renin.

In the rabbit the position is less secure, and evidence both for and against the participation of renin in the hypertension has been produced.

It seems improbable on general grounds that the hypertension produced by the same procedure and followed by similar effects on heart and arteries should differ essentially in mechanism in the two species. The hypertension following renal artery constriction in the rabbit is probably not of nervous origin, for in unpublished experiments we have found that it continues when all the visible nervous strands are removed from the renal pedicle (6 animals), and we have previously found in two animals that the temperature difference between normal and sympathectomised ears is unaltered by the development of hypertension (24). The time that elapses between the removal of the ischæmic kidney and the regain of normal pressure is in favour of a chemical and against a nervous origin for the hypertension; it is similar in rabbit (13) and dog (1). Lastly, Hill and Pickering (13) have shewn that a sustained hypertension may be produced in the unanæsthetised rabbit by a continuous infusion of renin provided the dosage is kept small, and that the hypertension resembles experimental renin hypertension in its intensity, in the behaviour of the ear vessels and in the time course of the hypertension following the end of the infusion of renin in the one case and the removal of the ischæmic kidney in the other.

On the other hand, Prinzmetal, Lewis, Taggart, Wilkins and Drury (28) have found the arterial pressure of nephrectomised or normal rabbits unaffected by transplanting kidneys with their renal arteries constricted; nor did they observe a rise in arterial pressure on releasing the circulation

to a completely ischæmic kidney even in unanæsthetised animals. From experiments in which renin was injected or infused into normal and hypertensive animals, Taggart and Drury (31) conclude that renin cannot be the cause of experimental hypertension in the rabbit. They point out that while many of their animals with hypertension had arterial pressures greater than 160 mm. Hg, they were unable to maintain pressures over 120 mm. Hg with renin; that while the animal with experimental renal hypertension responds to renin as least as well as the normal animal, the animal having a renin infusion does not; and finally when tachyphylaxis is produced in experimental hypertension the arterial pressure is not reduced. These are cogent arguments and require careful consideration.

Although experiments in the dog have not been made with the precise purpose of those of Taggart and Drury, it seems that similar phenomena may occur. Thus Corcoran and Page (4) have succeeded in maintaining the arterial pressure of dogs raised by 18 to 20 mm. Hg with infusions of renin; with higher rates the arterial pressure is not maintained. Again Page has found the dog with hypertension due to perinephritis responds to renin, either as a single injection or as an infusion, at least as well as the normal animal (21). In the phenomena to which Taggart and Drury have drawn attention it is therefore improbable that the rabbit differs from the dog.

We can confirm Taggart and Drury's statements for the rabbit, except for the effect of renin tachyphylaxis on hypertension, which we have not tried. But while we have had rabbits with experimental hypertension with pressures of over 150 mm. Hg and have never been able to maintain a pressure of over 112 mm. Hg by infusing renin, these are by no means comparable instances, for they differ in time. In our experience the higher pressures found in experimental hypertension are not reached until some weeks have elapsed; during the 12 hours following renal artery constriction the highest pressure we have observed has been 120 mm. Hg (in the central artery of the ear). The duration of the hypertension in Taggart and Drury's experiments is not stated, but for the reasons given we suspect it may have been a matter of weeks rather than hours. And we think that while their arguments do not finally exclude the intervention of renin in renal hypertension, they suggest that in chronic hypertension other factors contribute to the rise of pressure.

The observations described in this paper may be viewed against this background. Our failure to recover renin from the blood in experimental hypertension is not to be regarded as evidence against the renin hypothesis. For our animals had hypertension of several months duration and while we have succeeded in recovering renin from the blood of animals with pressures raised to a comparable extent by renin injected 10 min. previously, the two instances are not truly comparable. It is possible that with more sensitive methods applied to the renal vein blood a positive answer would be obtained.

Observations on the renin content of the kidney cannot provide convincing evidence for or against the renin hypothesis, for while the amount in the kidney depends on the rates of production and loss of renin, it measures neither. Nevertheless the changes in renin content demonstrated in animals with recent hypertension indicate that renal artery constriction does affect one or both of these processes, and in this sense the results support the renin hypothesis. Thus, when renal artery constriction is severe enough to cause renal necrosis, the appearance of hypertension which is not maintained is readily correlated with the observed exhaustion of the kidney's renin. Again the maintained hypertension which accompanies a less severe constriction and a macroscopically normal kidney is compatible with the observed increased renin content of the kidney. It seems unlikely that the onset of hypertension after constricting the renal artery is unrelated to changes in the renal content of renin, particularly when the course of the hypertension falls so clearly into line with the observed decrease or increase in the kidney's renin.

In chronic hypertension the renin content of the kidney is essentially normal, and thus differs sharply from the position at an earlier stage. It is of course conceivable that in such animals the rate of formation of renin is so nicely adjusted to the rate of release as to leave the renal content unchanged, but this does not appear very probable, and it is worth while considering the possibility already, as we think, raised by the experiments of Taggart and Drury that factors other than enhanced secretion of renin materially contribute to hypertension in this stage. Apart from any question of abnormal secretion of renin, both anatomical and chemical changes have been shewn to accompany prolonged experimental renal hypertension. Thus the heart hypertrophies (24) and in severe hypertension arteriolar necroses (8, 33) are found. Although hypertrophy of the heart may enhance the responsiveness to a pressor agent it is unlikely to be more than a minor factor in maintaining the hypertension. Acute arteriolar necrosis may be absent in animals with prolonged hypertension and normal renal content of renin as Table I shows. Page (14) has shown that the plasma constituent on which renin acts becomes increased in animals with renal hypertension; but while this would enhance the action of any renin secreted, we cannot assume that it could alone account for the hypertension unless further evidence is forthcoming. The suggestion put forward must remain purely speculative until the factors concerned are identified. It would, however, be interesting to know to what extent in prolonged hypertension in the rabbit the raised pressure is dependent on the presence of the ischaemic kidney; our data on this question are as yet too incomplete to mention.

We had hoped that the assays might suggest a method of identifying such forms of human hypertension as are similar in mechanism to experimental renal hypertension. This hope has not been realised, since we have not detected any abnormal renin content of the tissues in prolonged

experimental hypertension to which most forms of human hypertension are most comparable.

SUMMARY.

1. Extracts of rabbit kidneys made by three different methods have been assayed for their renin content by finding the amount raising the unanæsthetised rabbit's blood pressure to the same extent as a given dose of a standard preparation. The standard was stable for at least 2½ years.

2. The renin content of normal rabbits' kidneys shows considerable variations, the causes of which are undetermined, although higher values tend to occur in rabbits that are immature than in those that are fully grown.

3. When hypertension is produced by constricting the renal artery in the rabbit, the renin content of the kidney depends on the degree of constriction and on the duration of the hypertension. When the constriction is severe enough to produce renal necrosis, the hypertension is fleeting, and after 1 to 2 days the renal renin content is abnormally low. When the constriction is severe enough to produce hypertension within 12 hours but not to produce renal necrosis, the hypertension is maintained and in the first 8 days the renal renin content tends to be abnormally high. When hypertension has lasted 2 to 17 months the renal renin content is normal.

4. By a method of extraction described in the text renin can be recovered from blood 10 to 15 min. after its intravenous injection. None has been recovered from the blood of rabbits with hypertension of 2 to 5 months duration.

5. The results obtained are consistent with the hypothesis that the hypertension which occurs in the first few days after constricting the renal arteries is due to the release of renin from the kidney. The normal renal content of animals with prolonged hypertension suggests that factors other than the enhanced secretion of renin may contribute materially to the genesis of prolonged hypertension after renal artery constriction. These factors have not yet been identified.

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EXPERIMENTAL TRINITROTOLUENE POISONING; THE EFFECT OF DIET.

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Trinitrotoluene (T.N.T.) was considered an innocuous substance until experience during its manufacture on a large scale in the last and the present war revealed the contrary. Estimates of the disability arising amongst munition workers from exposure to T. N. T. vary, but a recent communication (24) states that 25% of the total strength of the workers have symptoms referable to this cause. The degree of illness varies. In Swanston's series of 495 cases, 15 were regarded as seriously ill, and of these 6 died (24). But the serious nature of the problem cannot be judged by the incidence of fatal cases. Loss of working time occurs in most cases and transference of the worker to another occupation is advisable in a considerable proportion. Any measures therefore, which throw light on the factors which influence susceptibility to T. N. T. poisoning are of importance.

Excellent clinical accounts of T. N. T. poisoning in munition workers have been given by O'Donovan (17) (19) and Lane (11). Their accounts concern workers sufficiently ill either to report sick or to attract the attention of the inspecting medical officer. The main lesions reported by them are dermatitis, cyanosis, "gastritis," toxic jaundice and aplastic anaemia. A general survey of the workers in a munition factory in America showed, however, that symptoms attributable to exposure to T. N. T. but insufficient to excite complaint, were present in 72% of the workers (27). Of the lesions due to T. N. T. dermatitis, cyanosis and "gastritis" are common but not fatal; toxic jaundice and aplastic anaemia are infrequent but often fatal. It is natural, however, that considerable attention should have been paid to the fatal complications and that attempts to reproduce them experimentally should have been made. Several workers have reported the experimental production of hepatic lesions but these have been slight, infrequent and uncharacteristic. The position with regard to aplastic anaemia is equally unsatisfactory for, with the exception of one cat reported by Pantou (21), it has not been observed in animals receiving T.N.T.

The results reported in this paper show that the susceptibility to T. N. T. is modified by the nature of the diet and that typical necrosis of the liver and a profound, though not aplastic, anæmia can be produced in rats by T. N. T. when the animals are given a diet rich in fat.

METHODS.

Rats. White "Wistar" rats were used. Each batch was given the stock diet for at least a fortnight before being used for any experiment. In each experiment all the rats in each group were of the same sex and of approximately the same weight. Control groups of animals were used in every experiment. In the earlier experiments rats weighing 90 g. to 120 g. were used; in the later ones animals weighed about 160 g.. The rats of each group were kept in a single cage. At the same time each day, all the animals in each group were weighed together and the average weight of the rats thus determined.

Diets. The stock diet had the following composition: wheatmeal bread 75%, whole milk powder 10%, brewer's yeast 7.5%, scrap meat 7.5%. Half a c.c. of cod liver oil was added to each 100 g. of the diet and 5 g. of cabbage was given twice a week.

Three basic diets were used in this investigation and for convenience they will be referred to by the name of the food constituent which predominated in each.

Protein diet. Casein 60%, wheatmeal bread 34%, brewer's yeast 6%, 1 c.c. of cod liver oil was added to every 100 g. of the mixture.

Carbohydrate diet. Wheatmeal bread 90%, casein 5%, brewer's yeast 5%, 1 c.c. of cod liver oil to every 100 g. of the mixture.

Fat diet. Rendered bacon fat 50%, wheatmeal bread 36%, casein 8%, brewer's yeast 6%. 1 c.c. of cod liver oil to every 100 g. of the mixture.

Salt mixture (23) was added to the diet in a few of the experiments but its inclusion made no appreciable difference in the results.

*Feeding.** Weighed amounts of food were given at the same time each day. At the end of twenty-four hours the food remaining in the dish, and any which had been spilt, was collected and weighed. In the case of diets which required moistening the actual amount of food eaten was calculated from the weight of the residue of moist food by means of a factor arrived at by determining the proportion of moisture in the moistened and in the unmoistened food. Paired feeding was carried out in many experiments by giving to the control group the same amount of food per head as had been consumed by the animals of the experimental group in the previous 24 hours. Save in the case of paired feeding experiments an excess of food was always given. Water was not restricted.

* We are indebted to Dr. Charlotte Himsworth for help with the feeding of the animals.

T. N. T. In the majority of experiments crystalline commercial T. N. T. was used. In one set of experiments pure α T. N. T. was given but the results differed in no way from those obtained when the commercial preparation was used.*

The T. N. T. was administered in oily solutions. When the diet contained fat the amount of T. N. T. required was dissolved in part of the fat of the diet. When the diet did not contain fat the T. N. T. was dissolved in the minimum quantity of arachis oil and then mixed with the food. When T. N. T. was given parenterally or by stomach tube it was given as a 5% solution in arachis oil. Unless stated otherwise, the T.N.T. was given mixed with the food.

The dose of T. N. T. aimed at in the feeding experiments was 0.15 g. per kilo of rat each day. When T. N. T. was given by the stomach tube the exact dose was given; when the T. N. T. was mixed with food sufficient T. N. T. was given to ensure that the average amount consumed daily was 0.15 g. per kilo.

Qualitative tests for T. N. T. in faeces and organs were done by extracting the material with acetone, filtering and adding weak alcoholic potassium hydroxide to the filtrate. The presence of T. N. T., even in minute amounts, was shown by the development of a purple colour.

Haematological examinations.† Blood samples were taken from the tail. Hæmoglobin determinations were made against the Haldane standard. Within each experimental group the variations in hæmoglobin percentage were slight and we have, therefore, recorded in this paper only the average values for the different groups. Blood counts and differential counts were done in the usual way. The percentage of reticulocytes was determined by the wet method. Marrow smears were taken from the lower third of the femur. Blood and marrow smears were stained with Leishmann's stain.

Histological examinations. The animals were killed by stunning. Samples were fixed in 10% formalin or in Bouin's solution. The latter gave better fixation for liver tissue. Frozen sections were stained with Scharlach R for fat, and paraffin sections with hæmatoxylin and eosin. Samples of liver and spleen were always taken from the same part of the organ. Liver samples were taken from the middle of the large central lobe which overlaps the stomach; sections of the spleen were taken from the centre of the organ.

RESULTS.

The noxious effects of T. N. T. in rats.

When T. N. T. is administered to rats one of two entirely different pictures results, and these may be distinguished as acute and chronic poisoning.

* We are indebted to Dr. C. A. Henry of the Factory Department of the Ministry of Labour and to Colonel Johnson of Woolwich Arsenal for supplies of commercial T. N. T. and to Dr. C. Rimington of the National Institute for Medical Research for the supply of α T. N. T..

† We are indebted to Miss D. Harding for assistance with some of these examinations.

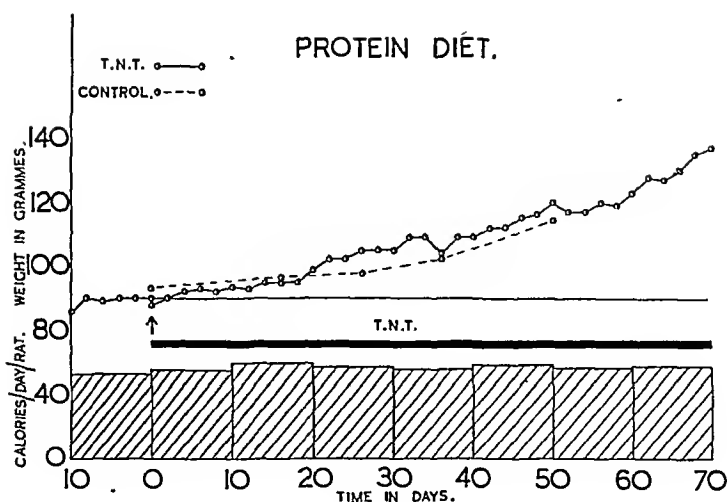


Fig. 1. Experiment showing that the administration of T. N. T. (0.15 g./kilo.) by mouth to rats on a protein diet causes neither loss of weight nor interference with the normal gain in weight on this diet. The food intake refers to the rats given T. N. T. and is expressed as the calorie value of the average amount of food consumed daily by one rat. The averages were taken over ten day periods.

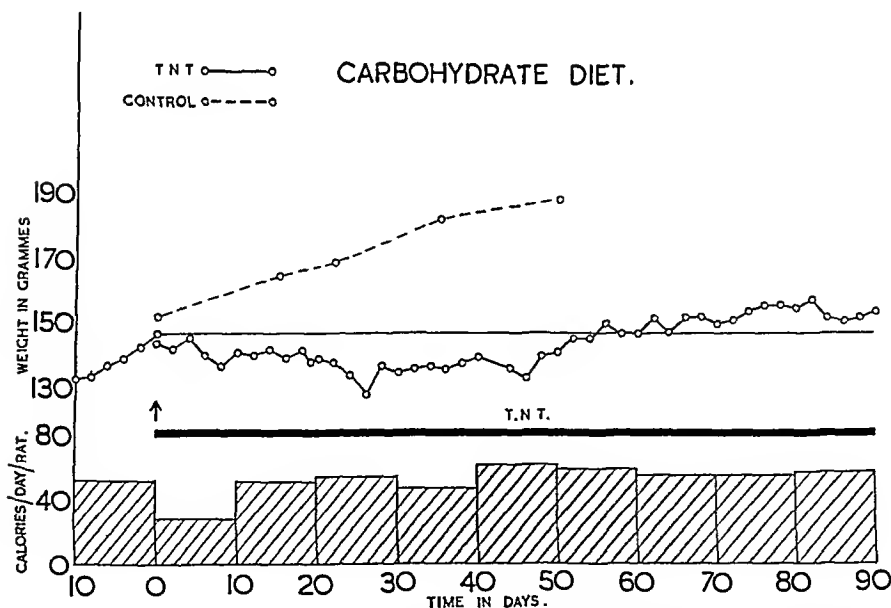


Fig. 2. Experiment showing that administration of T. N. T. (0.15 g./kilo.) by mouth to rats on a carbohydrate diet, whilst causing no significant loss of weight, prevents the increase in weight normally seen in rats on this diet. The food intake refers to the rats given T. N. T. and is expressed as the calorie value of the average amount of food consumed daily by one rat. The averages were taken over ten day periods.

If a large dose of T. N. T. such as 0.5 or 1.0 g. per kilo of rat is given subcutaneously, a characteristic clinical picture develops. Within a few minutes, as has been noted previously (16), a bright red pigment appears in the urine and in the space of an hour or so the rats appear gravely ill. They huddle in a corner of the cage, on being picked up they are limp and they pass little or no urine. This state continues for 48 hours or so when a proportion of the animals will have died and the rest be on the way to recovery. Death is preceded by a moribund state in which the animal lies limply in the cage breathing slowly and weakly. The skin is inelastic, indicating gross dehydration, and the animal is cold. Eventually it quietly dies. At autopsy the animals are found to be anæmic and dehydrated; the liver is small dark coloured and shrunken under the diaphragm; the spleen is greatly reduced in size and usually pink in colour. Sections of the liver show no evidence of fatty infiltration or necrosis and their most prominent feature is the small closely packed parenchymal cells in which the nucleus occupies most of the cell body. It is not our purpose to discuss this acute poisoning resulting from parenteral administration of T. N. T. Our object in mentioning it is first, because a certain number of animals receiving small repeated doses of T. N. T. orally die in this manner during the first week of T. N. T. administration, and second to point out that no trace of necrotic lesions in the liver has been found by us after acute poisoning from large single doses of T. N. T. administered parenterally.

The full picture of chronic poisoning differs markedly and it may be said at once such poisoning only develops in rats taking the fat diet. Little or no manifestation of poisoning has been seen in animals taking either the carbohydrate or the protein diet. When rats on a fat diet are given T. N. T. mixed with the food the following sequence of events occurs. Within half an hour the bright red pigment appears in the urine but the rats do not appear physically ill until a week or a fortnight after the beginning of the experiment. The physical state is then characterised by weakness and limpness and although, after some weeks, the rats may appear stronger and more active they do not regain the degree of health they possessed before the experiment began. About the third week the hair between the scapulæ begins to fall out and in experiments of long duration the animal may lose most of the hair on its body. During the first few days of the experiment there is a rapid loss of weight but this loss diminishes and the weight stabilises at a new and lower level. During the first week or so the loss of weight is associated with impaired appetite but later appetite returns and becomes greater than that of the control animals. Despite this the animals weight is not restored. Within the first two weeks of the beginning of T. N. T. administration an anæmia rapidly develops. The hæmoglobin concentration then declines more slowly. A week to a fortnight after commencing T. N. T. administration the liver lesions can be detected. Death, when it occurs, is usually within the first month or six weeks. Animals surviving beyond this time have been alive three months later.

The general outline of chronic T. N. T. poisoning, as it appears in rats, having now been outlined, special features of the condition, and the factors influencing their development can be considered in more detail.

The relation of diet to changes in body weight.

The effect of each of the three basic diets on the body weight of rats receiving daily 0.15 g. of T. N. T. per kilo. by mouth is shown in Figs 1, 2 and 3. Fig. 1 shows that when the animals receive a protein diet those given T. N. T. continue to increase in weight at the same slow rate as do the rats in the control group. Fig. 2 shows that the administration of T. N. T. to rats receiving the carbohydrate diet arrests the increase of weight. Fig. 3 shows that when T. N. T. is given to animals receiving the fat diet there is a rapid fall of body weight to a lower level and that this loss of weight is not subsequently made good.

The question now arises as to what these changes in weight are due to. Four possibilities suggest themselves; first that the change in the weight curve may be due to diminished intake of food; second that it may be due to some constituent of the diet combining with the T. N. T., and in proportion to the amount of the constituent present in the diet, to render T. N. T. relatively innocuous; third, that the amount of T. N. T. absorbed is dependent upon the constitution of the diet; and fourth that the different diets produce a real change in sensitivity of the animal to the poison.

Although there is often a decrease in appetite for some days after administration of T. N. T., whether this is given mixed with the food or by stomach tube, within a short time the animals are again eating normal amounts of food. Fig. 1 and Fig. 2 show that in the experiments where animals received protein or carbohydrate diets there was, after the first week, no diminution in the food intake. In the case of the rats on a protein diet the animals gained weight at the same rate as the controls (Fig. 1). In the case of animals receiving the carbohydrate diet, however, despite the sustained intake of food, the normal increase of weight was suspended (Fig. 2). Fig. 3 shows a paired feeding experiment on rats receiving the fat diet. It will be seen that the control group of rats increased in weight when taking the same amount of food upon which the rats receiving T. N. T. lost weight, and further that towards the end of the experiment the rats receiving T. N. T. were eating more food than the control rats and yet were failing to gain weight. (See also Table I). These experiments show that the failure to gain weight of the rats receiving T. N. T. and taking a carbohydrate diet and the loss of weight shown by the rats receiving T. N. T. and a fat diet are not due to a diminution of food intake.

Commercial T. N. T. consists of a mixture of the three isomers of this substance and it has been shown by Barger (1) that the β and γ isomers, but not the α isomer, combine with certain amino acids. It was thought possible that the failure of symptoms of T. N. T. poisoning to appear in rats

receiving a protein diet might be due to a combination of this type having occurred and being innocuous. This possibility was disproved by showing that when pure *a* T. N. T. was given to rats on each of the three basic diets the same variations in the severity of poisoning were seen as when commercial T. N. T. was used.

TABLE I.
Food consumption of the rats on the different diets.

No. of the 10-day period.	Protein diet + T. N. T.		Carbohydrate diet + T. N. T.		Fat diet (Control)		Fat diet + T. N. T.	
	Cal. rat/day	Cal. 100 g. rat	Cal. rat/day	Cal. 100 g. rat	Cal. rat/day	Cal. 100 g. rat	Cal. rat/day	Cal. 100 g. rat
1	55.2	59.3	28.4	20.3	39.4	25.8	39.4	28.8
2	59.6	61.4	52.8	37.2	46.1	28.8	46.1	34.6
3	57.6	55.2	55.2	40.9	42.5	25.6	42.5	33.2
4	57.2	53.5	48.0	35.3	37.1*	22.8*	42.2	34.8
5	58.8	50.7	63.4	46.3	46.7	28.9	45.4	38.1
6	57.6	47.6	59.2	33.0	48.0	28.2	57.2	48.0
7	56.8	42.7	55.2	36.5	46.1	27.6	64.6	55.2
8	—	—	55.2	36.1	47.4	26.8	59.5	48.0
9	—	—	57.2	37.1	42.4	24.3	63.7	51.7
10	—	—	55.2	35.2	46.1	26.3	60.2	50.0

* Fighting and losing blood.

Paired feeding of the rats on the fat diet given T. N. T. and the control group on the same diet was carried out until the excessive appetite of the former rendered it impossible. Before T. N. T. was given the rats on the protein diet each consumed food to the average daily value of 51.7 calories, or 57.4 cal./100 g. rat. Those on the carbohydrate diet, before receiving T. N. T. each consumed food to the average daily value of 52.4 calories, or 30.1 cal./100 g. rat. It will be seen that the effect of T. N. T. on food consumption was to cause a considerable increase in the case of rats on the fat diet, perhaps a slight increase in the case of rats on the carbohydrate diet, and no increase in the case of rats on the protein diet.

As T. N. T. is readily soluble in oil it might reasonably have been supposed that the effect of the fat diet in facilitating the appearance of symptoms of T. N. T. poisoning was due to the more complete absorption of T. N. T. from the fat diet. If this were so then presumably T. N. T. was incompletely absorbed on the protein and on the carbohydrate diets. Repeated analyses of the stools from animals on each of the three diets failed to show any trace of T. N. T. although similar analyses of rats faeces which had been ground up with traces of T. N. T. and either tested at once, or moistened and incubated for 24 hours at 37° C and then tested, gave strong positive reactions. The possibility of different rates of absorption of T. N. T.

being the explanation of the different degrees of poisoning seen when different diets were given is finally disproved by the following experiment. Two groups of six rats each were taken and the animals given a fat diet. The rats of one group were given 0.15 g. per kilo. of T. N. T. daily by stomach tube; the rats of the other group were given the same dose of T. N. T. subcutaneously. The animals in both groups showed the marked and rapid loss of weight which is usual in rats receiving T. N. T. whilst taking a fat diet. Two further groups of six rats were taken. One group received the carbohydrate diet; the other the protein diet. For the first fortnight the animals of each group were given 0.15 g. per kilo of T. N. T. daily by stomach tube. Thereafter they were given the same dose by subcutaneous injection. No loss of weight occurred in the animals of either group.

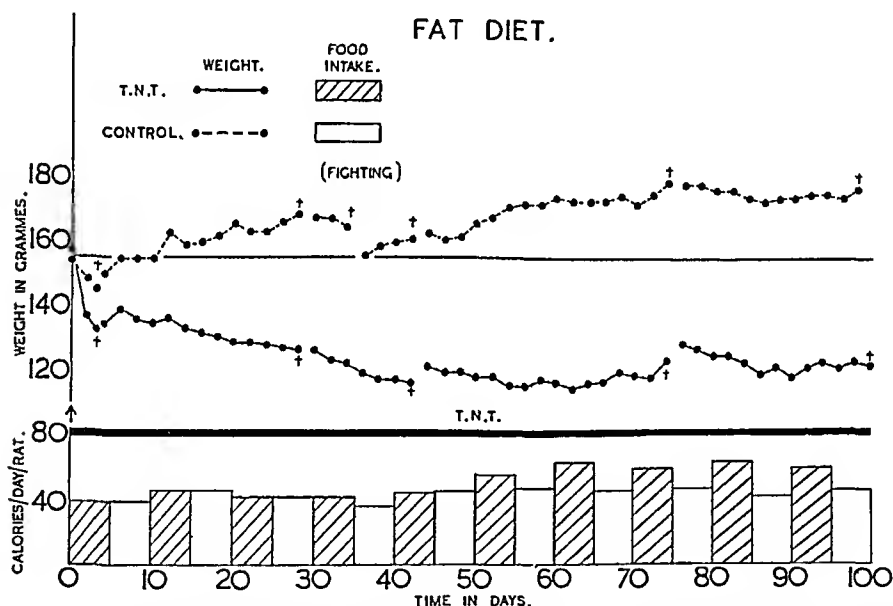


Fig. 3. Experiment showing that administration of T. N. T. (0.15 g./kilo.) by mouth to rats on a fat diet causes a rapid and sustained loss of weight. The food intake of the rats given T. N. T. is shown by shaded blocks, that of the control animals by unshaded blocks. The food intake is expressed as the calory value of the average amount of food consumed daily by one rat, these averages being taken over ten day periods. The experiment commenced as a paired feeding experiment but it will be seen that, after several weeks, the rats given T. N. T. ate more food than the control animals would eat.

It remains now to consider the fourth possibility, namely that the different effects of T. N. T. upon the body weight when the animals are receiving different diets are due to these diets altering the susceptibility of the animals tissues to T. N. T. There is positive evidence in favour of this view. It can be seen from Fig. 3 and from Table I that the food consumption of the animals on a fat diet receiving T. N. T. was greater than

that of the control animals and yet that their weight remained at a subnormal level. It will be seen from Table I that when rats are given T. N. T. it is only in the case of animals receiving a fat diet that this increase in the amount of food eaten is demonstrable. As there was no diarrhoea, nor any other evidence that the rats receiving T. N. T. were not absorbing the extra food ingested, this provides strong evidence that the metabolic rate of these animals was increased. In other words the fat diet enable T. N. T. to alter the metabolic processes in the animal's tissues.

The conclusion we draw from these experiments is that diminution in food consumption, formation of an innocuous compound by combination of T. N. T. with a dietary constituent, and variations in the completeness of absorption of T. N. T., cannot explain the different effects of T. N. T. on the body weight of animals receiving different diets, and that the evidence available indicates that these different effects of T. N. T. are due to the diet administered so modifying the animal's metabolism as to alter its susceptibility to poisoning by T. N. T. Of the three basic diets tested the fat diet is by far the most potent in rendering the animal susceptible to T. N. T..

Diet and the excretion of the abnormal pigments in the urine.

It was noted by Moore (16) that after administration of T. N. T. to rats a red pigment appeared in the urine. This observation has been confirmed by several observers including Voegtlin, Hooper and Johnson (27) and ourselves. The exact composition of this pigment is uncertain but it is undoubtedly derived from T. N. T.. Rats appear to be unusual in that they consistently pass this substance; cats, guinea pigs (16) and dogs (27) do not. A similar pigment is passed by rabbits only when very large doses of T. N. T. are given (16). Like human beings, however, dogs, cats and rabbits excrete in the urine a colourless derivative of T. N. T. which gives a purple or violet colour with the Webster test (28) (29). This chromogen has only been demonstrated in the urine and never in the blood or other tissues.

The relationship of the red pigment passed by rats to the colourless substance giving the Webster test is not established. A similar red pigment can be produced by shaking T. N. T. crystals with a solution of sodium carbonate in water, or more easily by dissolving the T. N. T. in acetone, making the solution alkaline with sodium bicarbonate solution and diluting with water. The small amounts of alkali required to produce these reactions in vitro suggested the possibility that in rats the urine as formed might contain a colourless precursor of the red pigment and that, as a consequence of change in reaction in the urine after passage, the red pigment is formed. That this is not so is shown by the observation that the red pigment has been noted in acid, in alkaline and in neutral urines, that alteration of the reaction of the urine within physiological limits does not alter the intensity of the colour, and that we have noted, as Moore (16) had previously, that the urine in the rat's bladder is red. Despite the fact that in rats receiving

T. N. T. the urine in the bladder is red we have never seen any trace of red pigment in the plasma or other tissues.

That the red pigment may have a similar significance to the Webster chromogen is suggested by a clinical observation. Observations on T. N. T. workers with toxic hepatitis have shown that the excretion of Webster chromogen decreases or disappears when toxic jaundice supervenes (9) (6). The rat with the most marked acute necrosis of the liver excreted a urine devoid of red pigment at the time of death. This absence of pigment is such an unusual finding that it is noteworthy.

The concentration and the quantity of red pigment excreted by rats receiving T. N. T. in the food are dependent upon the nature of the diet given. Rats receiving a carbohydrate diet excrete a urine which at most contains traces of a pink colour; rats receiving a protein diet excrete a pink urine; rats receiving a fat diet excrete a urine which looks like red ink. These estimates are based on the colour of urine collected separately not on staining of the animals fur or food which may give a mistaken impression. The volumes of urine passed on the three diets differ, the rats on the fat diet passing least. Nevertheless if the urines are all diluted to the same volume it will be found that the urine from the rats on the fat diet is still the redder. It thus appears that after administration of T. N. T. rats on the fat diet pass most red pigment, rats on the protein diet pass much less, rats on the carbohydrate diet pass only traces.

The close relationship between the amount of fat in the diet and the concentration of red pigment in the urine is shown by the following experiment. Two groups of rats were given T. N. T. and a carbohydrate diet. One group was kept as a control and in the other group fat was substituted for carbohydrate in the diet by successive stages, first 7% of fat, then 14% of fat, then 25% fat and finally 50% fat. The red colour of the urine deepened with each increase in the proportion of fat while the control group of animals continues to pass only traces of red pigment.

The dependence of the excretion of red pigment on the diet is also shown by the following experiment. Three groups of four rats were taken, each group was on one of the three basic diets, and each rat was injected subcutaneously with a dose of T. N. T. in oil equivalent to 0.2 g. per kilo.. During the first twelve hours all passed deep red urine. Five days later the urine from the rats on the protein diet was normal; two days later the urine from the rats on the carbohydrate diet became normal but it was not until twelve days after the injection that the rats on the fat diet ceased to pass red urine.

The excretion of red pigment is also dependent upon the amount of T. N. T. given. In the above experiment it will be noted that after a dose of 0.2 g. per kilo. all rats, irrespective of the diet they were taking excreted deep red urine. If a dose of 0.01 g. or T. N. T. per kilo. is given to three similar groups of rats no red pigment is excreted by the rats on the fat and carbohydrate diet but a trace is excreted by the rats on the protein diet.

The rats on the protein diet always develop a marked diuresis. In the above experiment the trace of red pigment excreted appeared in the first few hours. We consider it possible that the diuresis washed the red pigment out of the body before it could be further changed.

The above experiments throw some light on the way in which the body deals with T. N. T.. The fact that, when the dose is small enough, the rats on the fat and on the carbohydrate diet excrete no red pigment, yet when the dose is large they both excrete the red pigment, suggests that the pigment is formed at an early stage in the alteration of T. N. T. to a colourless compound and further that with large doses of T. N. T. the red pigment is produced in greater quantities than can be dealt with, so that some escapes from the body, while with small doses the body is capable of dealing with the quantities formed. In support of this suggestion is the observation that when the red pigment, prepared by the action of alkali on a solution of T. N. T. in acetone, is injected subcutaneously into rats in doses equivalent to 0.01 g. per kilo. no red pigment appears in the urine but if it is injected in doses of 0.1 g. per kilo. a typically red urine is excreted. The relatively early disappearance of the red pigment from the urine of rats injected with large doses of T. N. T. and receiving the protein or the carbohydrate diet, and the prolonged excretion of the pigment by rats given a fat diet, suggest that rats on a fat diet deal with T. N. T. more slowly than rats on either a carbohydrate or protein diet.

The red pigment is not the only one passed in the urine of rats receiving T. N. T.. When rats on a carbohydrate diet are given T. N. T. the urine colour changes from the normal pale yellow to a deep brown colour. That this brown pigment is also present in the urines of rats receiving either a fat or a protein diet can be shown by shaking the urine with amyl alcohol which extracts the red and thus reveals the presence of the brown pigment. It is possible that the brown pigment represents a further stage in the changes which T. N. T. undergoes in the body for when urine containing red pigment is allowed to stand or is heated in a water bath or is made strongly alkaline the red colour changes to a deep brown.

The relationship of excretion of the two kinds of pigment to the clinical state is clear. Rats excreting the brown pigment appear healthy; rats excreting the red pigment in high concentrations are ill and show the pathological changes of chronic T. N. T. poisoning. These observations suggest the possibility that the red pigment is itself more toxic than T. N. T.. Moore (16) administered the red substance formed by the action of alkalis on T. N. T. to cats and rabbits and concluded that it is as toxic as T. N. T.. We have found with rats that a red pigment prepared in the same way is, in doses up to 0.1 g. per kilo., no more toxic than T. N. T..

Our general conclusion with regard to the excretion of the red pigment in the urine of rats undergoing chronic poisoning with T. N. T. is that the concentration in which it is excreted is proportional to the fat content of the diet and is an index of the severity of the poisoning. Our experiments do

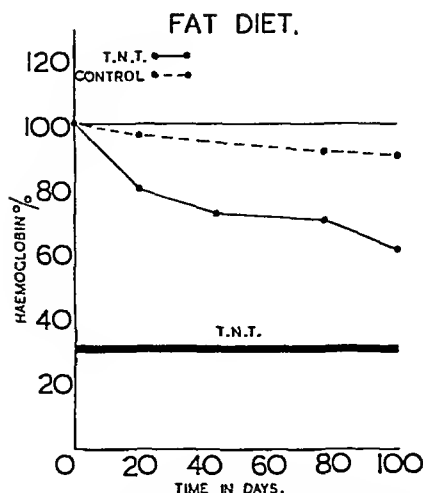


Fig. 4. Experiment showing the effect of T. N. T. administration (0.15 g./kilo.) on the haemoglobin concentration in the peripheral blood of rats given a fat diet. The control rats show a slight fall, the rats given T. N. T. show a rapid fall with no recovery.

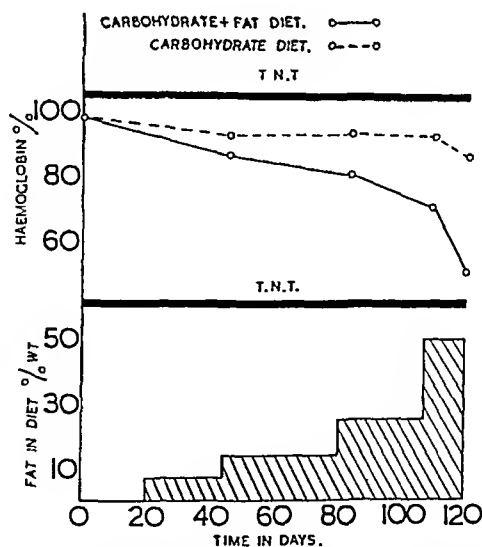


Fig. 5. Experiment showing the change in haemoglobin concentration in the peripheral blood in two groups of rats both receiving T. N. T. (0.15 g./kilo.), but one group of which was given the carbohydrate diet throughout the experiment (carbohydrate group) whilst the other received equicaloric amounts of diets containing progressively greater amounts of fat (carbohydrate and fat diet). It will be seen that with each increment of fat in the diet the haemoglobin value falls. *The decrease in the last value for haemoglobin in the animals on the carbohydrate diet can be attributed to their having suppuration in the tips of the tails.

not indicate that the pigment itself is significantly more toxic than T. N. T.. We consider it rather as a substance formed whilst T. N. T. is being disposed of by the body and that, with the doses of T. N. T. used in the chronic poisoning experiments, the escape of the red substance in the urine indicates that the disposal of T. N. T. is proceeding slowly and that the tissues are being exposed to a sustained high concentration of T. N. T. itself, or its derivatives. There is one significant exception to the conclusion. The red pigment was absent from the urine of the rat with the most severe liver damage, just as the Webster test is negative in the urine of patients with toxic jaundice due to T. N. T..

Changes in the blood system.

Hæmoglobin. The changes in the hæmoglobin concentration of rats receiving T. N. T. depends upon the diet they are given. Thus in an experiment with the protein diet on the 45th day the control group of rats had an average hæmoglobin value of 113%;* on the same day the group receiving T. N. T. had a hæmoglobin value of 104% and on the 113th day of the experiment the value was 105%. In an experiment with the carbohydrate diet the control group on the 46th day had an average hæmoglobin value of 99%* while the group receiving T. N. T. averaged 93% and on the 109th day 93%. In contrast the rats on the fat diet developed a marked anæmia. In one experiment, the hæmoglobin value of the control group fell slightly from an average of 100% on the first day to 90%* on the 100th day, while the value in the group receiving T. N. T. fell from an average of 101% on the first day to 62% on the 100th day (Fig. 4). It will be seen from these figures that T. N. T. can be administered to animals receiving the protein or the carbohydrate diet without causing anæmia but that when T. N. T. is given to rats on a fat diet anæmia develops.

In Fig. 4 are charted the changes in hæmoglobin value for two groups of rats undergoing paired feeding on the fat diet. One group served as the control; to the other T. N. T. was given in the food. It will be seen that T. N. T. at first rapidly lowers the hæmoglobin concentration, and that later the decrease is slower. The hæmoglobin values invariably decrease in this manner when T. N. T. is given, whether by subcutaneous injection, by stomach tube or in mixture with the food.

Fig. 5 shows the dependence of the fall in hæmoglobin percentage upon the amount of fat in the diet. With each successive increase of fat a further fall in hæmoglobin occurred. It would thus appear that a fat diet facilitates the action of T. N. T. in reducing the percentage of hæmoglobin in the circulating blood.

* It will be noted that the hæmoglobin values differ for the control groups on each of the three diets and we have invariably found in control animals that the hæmoglobin value was highest in those on a protein diet, lowest on a fat diet, and of intermediate value on a carbohydrate diet.

Red blood cells. In general it may be said that the changes in the circulating red cells which occur in chronic T. N. T. poisoning include an increase in the numbers of polychromatic cells and of reticulocytes and the appearance of numbers of nucleated red cells which are often themselves polychromatic. These changes reflect the nature of the diet, being most marked in the animals receiving T. N. T. and taking the fat diet, and moderate in those on a carbohydrate or protein diet. The degree of change can be indicated by the findings in two groups of animals on the fat diet, one a control group and the other receiving T. N. T.. Polychromasia was slight or absent in all four members of the control group and the average number of nucleated red cells was 0.25 per 100 white cells. In the five animals receiving T. N. T. polychromasia was conspicuous in each case and the nucleated red cells average 2.4 per 100 white cells. A reticulocyte count from a control rat showed 3.1% of reticulocytes and from a rat receiving T. N. T. 18.8% of reticulocytes.

White blood cells. Rats receiving a fat diet, irrespective of whether they received T. N. T. or not, showed a leucocytosis as compared with animals on protein or carbohydrate diets. The rats on the fat diet receiving T. N. T. however, in contrast with the controls and rats on other diets, showed a relative polymorphonucleosis. Thus a group of control rats on a fat diet showed an average of 22% of polymorphonuclear cells and 69% of lymphocytes—which accords with normal figures for rats—but rats on the same diet and receiving T. N. T. showed an average of 51% of polymorphonuclear cells and 41% of lymphocytes.

Bone marrow. Smears of the bone marrow from control rats shewed that, though the carbohydrate and protein diets produced no constant change from the normal, the fat diet resulted in more fat accumulating in their marrow, thus causing a reduction in its cellularity. Administration of T. N. T. to rats on a fat diet produces a definite erythroblastic hyperplasia, in which up to 40% of the cells seen may be erythroblasts, and the disappearance of fat from the marrow and its replacement by marrow cells. These changes are less marked, or absent, in rats receiving the protein or the carbohydrate diet. In two animals given T. N. T. and taking the fat diet the marrow was hypoplastic. In one only 3% of the cells seen were erythroblasts, as compared with normal count of up to 20%; in the other practically no cells were seen, the marrow appearing as an empty reticulum. These two exceptions are noteworthy because both animals also showed acute necrosis of the liver.

*Iron content of the spleen.** The amount of iron in the spleen varied with the diet even when the animals were not given T. N. T.. On the protein diet the control animals showed more iron than the control animals

* During the course of our experiments "wheatmeal" bread came into use and was used in making up the various diets. Prior to this white bread had been used. When the rats were receiving white bread little iron could be found in their spleens; after they were given diets containing wheatmeal bread considerable amounts of iron were always found in this organ.

on either the fat or the carbohydrate diets. But on each diet the rats receiving T. N. T. showed more iron in the spleen than the controls did.

The following conclusions appear justified by the above investigations on the changes in the blood and related tissues. Chronic T. N. T. poisoning in rats produces a fall in the hæmoglobin content of the circulating blood, an erythroblastic reaction of the bone marrow associated with the appearance of normoblasts, reticulocytes and polychromatic cells, and also of polymorphonuclear cells in the peripheral blood, and an increased deposition of iron in the spleen. The fall in hæmoglobin is conspicuous in rats on a fat diet but slight or absent in animals on a protein or carbohydrate diet. The changes in the blood cells, the marrow and the spleen are in general more pronounced in rats on a fat diet but also occur to a lesser extent in animals on carbohydrate and protein diets.

Liver changes.

Changes in the liver, attributable to administration of T. N. T., were demonstrated in all animals on the fat diet save those dying in the first ten days with symptoms of acute poisoning. No changes of any kind were demonstrated in animals receiving either the protein or the carbohydrate diet. The changes noted were of three kinds; fatty infiltration of the liver cells encircling the central veins, necrosis or disappearance of the cells round the central veins and proliferation of the Küpfer cells, and acute necrosis of a wide zone of cells round the central vein. Fig. 7 is a micro photograph of a section of the liver from a rat taking the fat diet and given T. N. T. and it shows the distribution of fat in the liver lobule. Fig. 6 shows a section of the liver of a control rat in the same experiment. Two features are evident from comparison of the two sections, first, that the liver from the animal given T. N. T. contains much less fat than does that from the control animal, second that, although in the control animals the fat is evenly distributed throughout the cells of the liver lobule, in the animals receiving T. N. T. the accumulation of fat is confined to a zone of cells around the central vein, or more accurately those farthest from the portal tracts. These differences are constantly found. By the end of the second week of T. N. T. administration fatty infiltration of the central lobular cells is evident, and, in animals showing no other histological changes, it is found in the same distribution and amounts up to periods of 99 days of T. N. T. administration. In the control animals, on the other hand, the amount of fat in the liver increases with the duration of fat administration. During the first month fat is seen distributed in fine droplets in the liver cells throughout the lobule being concentrated, if anywhere, round the portal tracts. After three months of a fat diet all the liver cells are distended with fat. For example 98 days after the fat diet was begun the liver of the control animal contained 16.6% of fat whilst the liver of the rat which had received T. N. T. throughout the

period contained only 5.8% of fat. We are, of course, aware that fatty infiltration of the central cells of the liver lobule is a common finding in many conditions of the liver and particularly after administration of liver poisons. We attach significance to it in these experiments because it was seen only in animals which had received T. N. T. and, because it is an entirely different picture from that seen in animals taking an identical diet without T. N. T.. In our opinion it is evidence of a mild degree of poisoning and we base our estimate of its mildness on the absence of necrotic lesions.

Fig. 8 and 9 illustrate typical histological findings in a liver showing the second type of lesion. Livers from control animals, killed at the same time, had appearances similar to Fig. 6. The low magnification, Fig. 8 shows the absence of parenchymal cells round the central veins. The high magnification, Fig. 9, shows that the centre of the liver lobule is occupied by a mass of Küpfer cells, some polymorphs and widely dilated sinusoids. These findings together suggest that the central liver cells have been killed and removed and their place taken by swollen Küpfer cells and dilated sinusoids. This interpretation is the same as that put forward by Cameron and Karunaratne (3) in explanation of the histological appearances in the liver three days after carbon tetrachloride poisoning. These workers traced the development of this appearance step by step from an initial central necrosis. Because of the evidence that liver cells have disappeared we regard this lesion—central collapse—as evidence of more severe damage to the liver than that indicated by central fatty infiltration.

In Fig. 10 and 11 microphotographs of sections from livers showing the third type of lesion are shown. It will be seen that around the central vein there is a wide zone of liver cells showing acute necrosis. Intravascular clotting is also present in the central veins. Around the portal tracts the liver cells appear healthy and they contained some fat whilst the necrotic cells contained none. This change has been produced in four out of twenty-five animals and there can be little doubt that this lesion indicates a severe poisoning. It indicates a relatively recent lesion for, as Cameron and Karunaratne (3) have shown, and we have confirmed, dead liver cells are rapidly removed. It is noteworthy that little evidence of regeneration was seen in sections showing either this or the previous lesion.

Macroscopically the liver of animals which have received T. N. T. for more than ten days always show a more or less pronounced reticular pattern but we have not found it possible to say from inspection of the whole organ whether central collapse or central fatty infiltration would be found on microscopic examination. In livers which show acute necrosis however the appearance is distinctive. The general mass of liver tissue is terracotta coloured and shows a clear reticular pattern; scattered on this general background are irregular yellow areas, varying from 1 to 3 mm. in length, which show acute necrosis on section. The distribution of these areas is not uniform. They are found in greatest numbers towards the free edge of the liver and in the omental and left lobes. It is interesting to recall that

Stewart (25) found that the areas of acute necrosis in the livers of munition workers dying from toxic jaundice due to T. N. T. showed a similar distribution. The colour of the liver of all animals on a fat diet was yellower than normal but the livers of animals given T. N. T. were the colour of terracotta, while the livers of control animals, after a prolonged period on the fat diet, were often the colour of butter.

Thus after administration of T. N. T. the severity of the liver damage varies in degree and it is clearly of importance to determine the factors which influence the severity of the lesion. Our results throw some light on this. Out of twenty rats taking the fat diet, which died or were killed later than nine days after T. N. T. administration was commenced, six showed fatty central infiltration, twelve showed central collapse and two acute necrosis. The two rats showing acute necrosis were killed on the 12th and 20th day respectively after daily T. N. T. administration was commenced, and in another group of seven rats, on a diet containing the same amount of fat but a greater proportion of protein, the two rats showing acute necrosis also died before the end of the third week. Of the twelve rats showing central collapse, eleven died or were killed between the 10th and 29th day after T. N. T. administration was begun. The 12th died on the 109th day. The significance of this last result is dubious as the rat had been ill for some time and the lesion was minimal. The six rats showing fatty infiltration were killed between the 30th and the 99th day. It thus appears that the severity of the hepatic lesion bears no relation to the total amount of T. N. T. given and that rats which survive 30 days of T. N. T. administration show only slight changes in the liver.

The point which does seem to be of importance in determining the appearance of severe lesions is whether or not the rats had been given the fat diet for some time before T. N. T. administration was begun. In the above group of 20 rats 5 were taken straight off the stock diet and started on T. N. T. and the fat diet simultaneously. Four showed fatty infiltration only and one—the ill rat dying on the 109th day—alone showed central collapse. Of the remaining fifteen all of which had taken the fat diet for a preliminary period, thirteen showed severe hepatic lesions. In this group two rats had received the fat diet for twelve days before T. N. T. was given; one showed fatty infiltration and one central collapse. Seven rats had taken the diet for twenty-eight days; six showed central collapse and one fatty infiltration. Six rats had received the diet for a preliminary period of one hundred days, none showed fatty infiltration, four showed central collapse, two showed acute necrosis. These results suggest that a preliminary period on a fatty diet predisposes rats to the development of severe hepatic lesions after T. N. T. administration.

One explanation is that, T. N. T. being readily soluble in fat and almost insoluble in water, a preliminary period on a fat diet might, by loading the liver with fat, favour the accumulation of T. N. T. in such high concentrations in the liver cells that they were killed. There are several points against this

suggestion. Subcutaneous injection of large doses of T. N. T. (0.5 g./kilo.) into rats which had taken the fat diet for sixty-six days caused no lesions at all in the liver by the time the animals were killed 3 days later. After administration of T. N. T. the amount of fat in the liver of animals, whose livers have been rendered fatty by previous administration of a fat diet, declines daily and by the time the hepatic lesions appear it is relatively small in amount. We have failed to detect T. N. T. in the liver of any rat.

The general conclusions from these results are first, that in the rat hepatic lesions due to T. N. T. only occur when the animal is taking a fat diet, and second that a preliminary period on a fat diet before T. N. T. administration is commenced appears to facilitate the production of severe hepatic lesions.

Loss of hair.

Loss of hair is a late development, appearing only after the animals have been receiving T. N. T. for several weeks. The baldness is only seen in rats receiving T. N. T. mixed with the fat diet. It is not seen in rats on a fat diet which receive T. N. T. by stomach tube. We conclude therefore that the loss of hair is a result of skin contamination by T. N. T. dissolved in the fat of the diet.

DISCUSSION.

It was noted by Moore (16) that rabbits, cats and, less constantly guinea pigs, lost weight when given large doses of T. N. T.. The same finding in the case of dogs is evident from the protocols of the paper by Voegtlin, Hooper and Johnson (27). Our results show that even with small doses of T. N. T. a loss of weight occurs in rats but only when they are given a diet which renders them susceptible to poisoning. We have been unable to find any observations on the changes in weight of human beings exposed to T. N. T..

Our figures for food consumption show that rats taking a fat diet and given T. N. T. develop an increased appetite after the lapse of several weeks when they are showing symptoms of severe poisoning. A similar increase in appetite was not seen after T. N. T. administration in the case of rats receiving either the protein or the carbohydrate diet (Table I); and these animals showed little evidence of poisoning. It seems, therefore, reasonable to suggest that the increased appetite is attributable to the T. N. T., and that in rats it is a symptom of T. N. T. poisoning. We know of no recorded observations of the food consumption of human beings exposed to T. N. T. but it is interesting to note that factory doctors report that workers with T. N. T. are well aware that their appetite is increased.

It has already been pointed out that a red pigment, such as is excreted after T. N. T. administration in rats, is not found in the urine of munition workers. Red staining of the skin in the axilla (21) or hair is, however, common in man, but, considering the ease with which red pigments are directly produced from T. N. T. by the action of alkali, this may indicate merely skin contamination.

It is generally believed that rats are the only animals which excrete the red pigment but when heavy doses of T. N. T. (0.5 g./kilo. upwards) are given to rabbits they also excrete a red pigment (16) (21). This observation appears significant in relation to our findings. Our results show that with moderate doses of T. N. T. (0.15 g./kilo.) by mouth rats on a fat diet excrete high concentrations of red pigment and show severe symptoms of poisoning, but that rats on a carbohydrate or protein diet excrete little and show few if any toxic manifestations; that with small doses of T. N. T. (0.01 g./kilo.) subcutaneously no red pigment, but with large doses (0.2 g./kilo. and upwards) much red pigment, is excreted by rats on any of the three diets. It thus appears that excretion of the red pigment after exposure to T. N. T. is not a species peculiarity but, both in rabbits and rats is governed by two variables, dosage and susceptibility. The rabbit is less susceptible and hence requires large doses of T. N. T.; the rat is more susceptible and requires only small doses. The fact that the susceptibility of the rat to T. N. T. can be influenced by the character of the diet suggests the possibility that similar modifications of diet may have similar effects in other species.

With the exception of the solitary experiment described by Panton (21) no worker has reported the production in animals of an aplastic anæmia similar to that seen in T. N. T. workers, and in the absence of further results we would not attach significance to our finding of hypoplasia of the bone marrow in two animals. Many workers have, however reported the development of another type of anæmia both in experimental animals and in workers with T. N. T.. Voegtlin, Hooper and Johnson (27) described the anæmia found on routine examination of workers in a T. N. T. factory as characterised by "a reduction in hæmoglobin percentage, the presence of anisocytosis and poikilocytosis, polychromatophilia, fragmentation of red cells, and the appearance of nucleated and reticulated red cells in the circulating blood." This condition they found in 72% of 237 workers and Minot (15), working at another factory, found a similar type of anæmia in 83% of the workers. Experimentally Moore (16) and Wyon (30) found that rabbits showed a fall in hæmoglobin and red blood cells after T. N. T., Voegtlin, Hooper and Johnson (27) produced in dogs blood changes similar to those they had observed in T. N. T. workers, and we have shown that similar changes occur in rats taking a diet which renders them susceptible to T. N. T.. In the last two investigations an erythroblastic hyperplasia of the bone marrow and increased deposition of iron in the reticulo-endothelial system was demonstrated in the animals. Turnbull (26) demonstrated siderosis of the spleen and Davie (7) hyperplasia of the bone marrow in human cases of T. N. T. poisoning. It thus appears that, with the exception of aplastic anæmia, lesions of the blood and associated tissues similar to those observed in human cases of T. N. T. poisoning can be produced in rats and that the liability to develop these changes is increased by giving a fat diet.

Clinically a variable leucopenia, leucocytosis, relative increase of polymorphonuclear cells or lymphocytes may occur (15) (27). It is therefore of interest that Voegtlin and his colleagues (27) find that T. N. T. poisoning is associated with a relative polymorphonucleosis in dogs, as we find it to be in rats.

The toxic jaundice seen in T. N. T. workers has certain peculiar features. The long latent period which may elapse between exposure to T. N. T. and the development of jaundice was early noted (17) (19). A similar phenomenon is shown in our experiments. Rats dying or killed within the first week of T. N. T. administration, whether the T. N. T. was given in small daily doses by mouth or as a single large subcutaneous injection, never showed hepatic lesions. The hepatic lesions all occurred in animals which had been receiving T. N. T. for nine or more days. It is evident therefore that the liver damage produced by T. N. T., unlike that produced by chloroform or phosphorous, requires time to develop. Another point of interest is the incidence rate of cases of toxic jaundice amongst T. N. T. workers. During the first two weeks of employment such cases are rare; later the incidence rises sharply to reach a peak at the third month, falls equally sharply to a low level at the sixth month and then remains at this low level (5). The fatality rate follows the incidence and severe cases are practically unknown after twelve months exposure to T. N. T.. In our rats liver damage was not found during the first week of T. N. T. administration; with one explicable exception all the cases of severe liver damage occurred between the 10th and 30th day; rats surviving beyond this period showed only mild lesions. This similarity is at least suggestive although we would add the caution that the rats surviving for long periods had not been on the fat diet for a preliminary period before T. N. T. was given.

This demonstration of a latent period in experimental T. N. T. poisoning is of theoretical interest. Several investigators (4) (18) have remarked on the apparent epidemics of toxic jaundice amongst T. N. T. workers and, associating this with the latent period, they have suggested, on the analogy of neoarsphenamine jaundice, that two factors are necessary for the development of the hepatitis (19). In some epidemics of neoarsphenamine jaundice there has been reasonable evidence that two such factors were involved, namely exposure to the drug and later to persons infected with "catarrhal jaundice" (12). Our experiments do not appear to admit the possibility of such a second factor and indicate that the latent period between exposure to T. N. T. and the development of hepatic lesions is a feature of the toxic action of T. N. T. itself.

Although several previous workers have reported that administration of T. N. T. to animals will occasionally produce slight hepatic damage none have claimed to have produced the acute necrosis seen in patients with toxic jaundice. Out of numerous experiments Moore (16) reported that after administration of 1.5 g. of T. N. T. to each of two rabbits fatty degeneration, with in one case necrosis of the centre of the hepatic lobule, was found.

Wyon (30) was unable to produce hepatic damage by chronic poisoning but in four out of fourteen experiments in which a large single dose was given fatty degeneration was seen. One of the four animals had had a previous injection of N.A.B. nineteen days previously, another was killed with chloroform. Panton (20) reported that in one cat of his series marked central necrosis and fatty infiltration of the liver lobule was found. This animal received subcutaneous injections of *B. coli* as well as T. N. T.. Voegtlin, Hooper and Johnson (27) state that slight fatty changes were occasionally found in their dogs but that acute necrosis of the liver was never observed. Descriptions of the appearance of the liver of human cases dying from toxic jaundice after exposure to T. N. T. have been published by Stewart (25) and by Turnbull (26). The microscopic changes they describe and illustrate closely resemble those we have found after giving T. N. T. to rats taking a fat diet. As well as the general structural resemblance there are two points of detail which reveal further similarity. In microscopic sections from the rats showing acute necrosis the amount of fat has been relatively small and absent from the necrotic areas. This appearance was considered by Stewart (25) and Turnbull (26) to be a noteworthy feature of the liver in human cases of toxic jaundice due to T. N. T. and is in marked contrast to the appearance in sections from cases, both clinical and experimental, of acute necrosis due to such poisons as chloroform and phosphorus. The second point of resemblance is the presence of intravascular clotting in the central veins. This was noted by Stewart (25) in human cases of jaundice due to T. N. T. and was present in our rats with acute necrosis (Fig. 10).

Thus the administration of T. N. T. to rats on a fat diet produces a condition the clinical and pathological features of which resemble closely those observed in T. N. T. poisoning in human beings, but its administration to rats on protein or on carbohydrate diets produces few if any toxic effects. In their investigations into T. N. T. poisoning Voegtlin, Hooper and Johnson (27) paid attention to the dietary factor and stated that in general animals fed on a bread and milk diet develop a more acute and severe anaemia than those on a mixed or meat diet. The occurrence of evident exceptions to this rule and the great individual differences in susceptibility of different animals, however, only allowed them to draw the conclusion that "The composition of the diet seems to be a factor influencing the susceptibility of the animals to T. N. T. poisoning." Nevertheless in the report of their field investigation they suggested that the absence of cases of severe T. N. T. poisoning amongst the workers in the factory they studied might be due to the good quality of the diet provided. The results which agree most clearly with ours are those of Messinger and Hawkins (13) on the effect of different diets in protecting dogs against neoarsphenamine poisoning. They have demonstrated clearly that in dogs taking a fat diet marked hepatic lesions develop whilst in dogs receiving protein or carbohydrate diets the lesions are slight.

Recently it has been shown that certain dietary factors may themselves cause liver injury. Liver injury has been produced in rats by means of a

high fat and low protein diet (2). The absence of liver injury in our control animals on the fat diet excludes this explanation of our results. Acute liver injury has been produced (10) (22) on diets poor in the vitamin B complex and has been prevented by addition of yeast to these diets. All our diets were rich in yeast and again the control animals showed no liver injury. Lastly there is the work of Miller and Whipple (14) showing that dogs deprived of protein until their plasma proteins fell developed hepatic lesions after administration of chloroform. The plasma proteins in three of our control rats on the fat diet were normal and no lesions developed in animals on the carbohydrate diet which contained slightly less protein than the fat diet. We consider therefore that the lesions demonstrated in our experiments are the result of T. N. T. poisoning and not the result of any dietary deficiency.

SUMMARY.

(1) The development of toxic manifestations in rats after administration of T. N. T. is dependent upon the nature of the diet. Severe symptoms and marked pathological lesions develop in rats taking a high fat diet whilst in animals taking a high carbohydrate or a high protein diet the ill effects are slight or absent.

(2) Chronic T. N. T. poisoning in rats shows the following features :—

- (a) Loss of weight.
- (b) Increased appetite.
- (c) Excretion of high concentrations of T. N. T. derivatives in the urine.
- (d) Changes in the blood and associated tissues the characteristics of which are a great decrease in hæmoglobin, the appearance of normoblasts, reticulocytes and polychromatic erythrocytes in the peripheral blood, an erythroblastic hyperplasia of the bone marrow and siderosis of the spleen.
- (e) Hepatic lesions which range from fatty infiltration to an acute necrosis of the parenchymal cells.
- (f) Loss of hair.

(3) Evidence is presented that the effect of the high fat diet in facilitating the toxic action of T. N. T. is due to the fat diet so influencing the animal's metabolism as to impede its ability to dispose of the T. N. T. within its tissues.

(4) The close resemblance between the effects of T. N. T. in the rat and the findings in human cases of T. N. T. poisoning is demonstrated.

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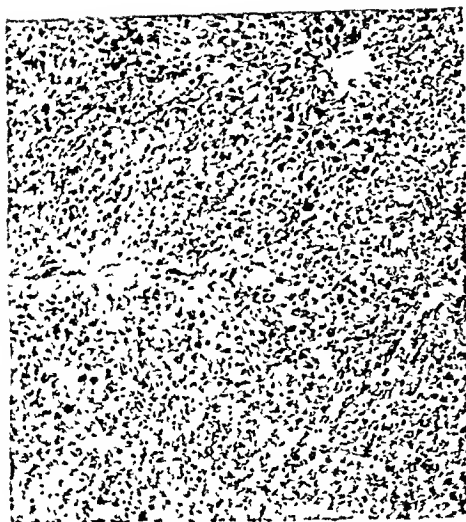


Fig. 6. ($\times 40$). Liver from control rat on fat diet, stained for fat, and showing that the fat is evenly distributed throughout the parenchymal cells. Scharlach R.

Fig. 7. ($\times 40$). Central fatty infiltration. Liver from rat on fat diet and given T.N.T. orally. The fat is present only in the cells round the central veins. Scharlach R.

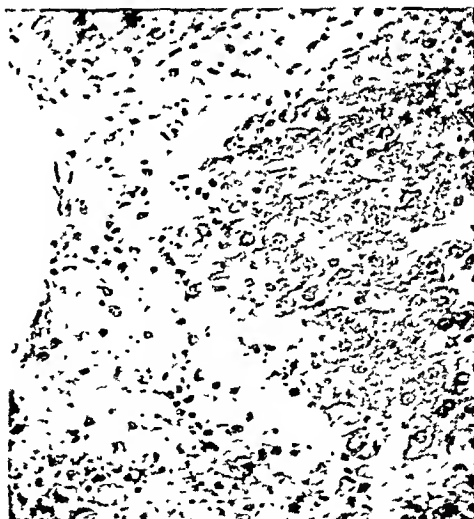
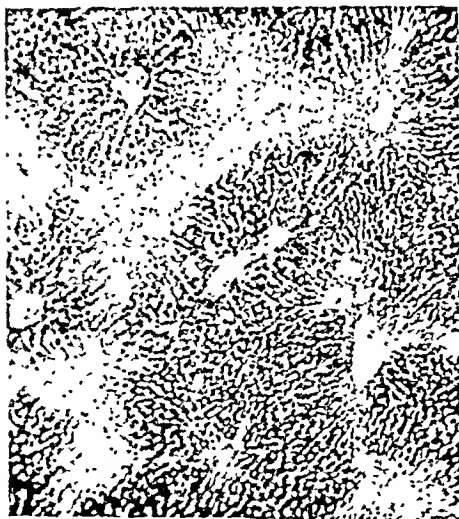


Fig. 8. ($\times 50$). Central collapse. Liver from rat on fat diet and given T.N.T. orally. The hepatic parenchymal cells have disappeared from around the central lobular veins. Haematoxylin and Eo-in.

Fig. 9. ($\times 200$). Central collapse. From same section as Fig. 8. The zone round the central vein, from which the liver cells have disappeared, is occupied by dilated sinusoids, swollen Kupfer cells and a few polymorphs.



Fig. 10. ($\times 50$). Acute necrosis. Liver from rat on fat diet and given T. N. T. orally. Normal liver cells remain only round the portal tracts. The remainder of the liver lobule is occupied by necrotic cells and debris. Intramural thromboses are present in the central vein. Hæmatoxylin and Eosin.

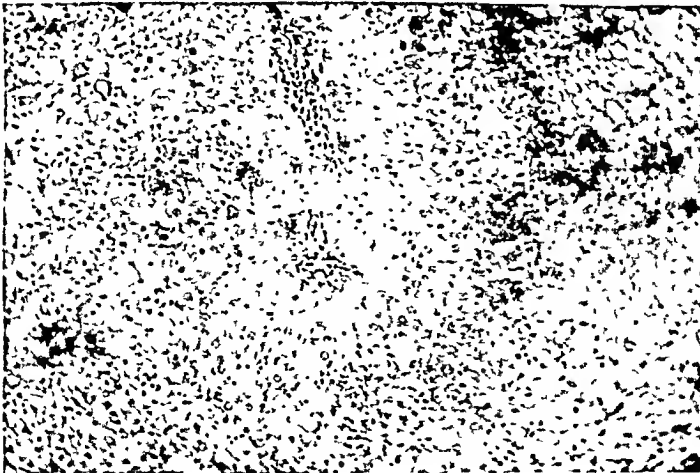


Fig. 11. ($\times 120$). Acute necrosis. From same section as Fig. 10. Around the portal tract is a narrow zone of normal parenchymal cells. Outside these is a zone of deeper staining cells which are necrotic and beyond there remains only the hepatic reticulum with the debris of dead parenchymal cells.